Journal of Clinical Virology 45, S1 (2009) S55–S61

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Clinical performance of the APTIMA® HPV Assay for the detection of high-risk HPV and high-grade cervical lesions

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ARTICLE INFO

Keywords:
HPV
mRNA
Clinical sensitivity
Clinical specificity

ABSTRACT

Background: Human papillomavirus (HPV) DNA testing is widely used in conjunction with Papanicolaou (Pap) testing in cervical cancer screening programs to improve the detection of high-grade lesions. While HPV DNA test sensitivity is good, an improvement in specificity is desired. Detection of HPV mRNA may improve specificity. The APTIMA® HPV Assay detects the mRNA of 14 high-risk HPV types in liquid-based cytology specimens.
Objective: To evaluate APTIMA HPV Assay performance for detection of high-risk HPV and high-grade cervical intraepithelial neoplasia (CIN) compared to Qiagen's Hybrid Capture 2 HPV DNA (HC2) test.
Study design: Liquid Pap specimens were collected from 800 women referred to colposcopy and tested with the APTIMA HPV Assay and the HC2 test. Complete results were available for 753 subjects. A subset of samples (n = 393) were typed using Roche's Linear Array HPV Genotyping Test.
Results: Sensitivity and specificity for detection of high-risk HPV were >92% and 99% for the APTIMA HPV Assay and 93% and 82% for the HC2 test. Clinical sensitivity and specificity were 91% and >55% for detection of CIN 2+, and 98% and 53% for detection of CIN 3+ for the APTIMA HPV Assay; values for the HC2 test were 95% and 47% for CIN 2+, and 99% and 44% for CIN 3+.
Conclusions: The APTIMA HPV Assay is sensitive and very specific for detection of high-risk HPV. The APTIMA HPV Assay had similar clinical sensitivity for disease detection but higher clinical specificity than the HC2 test, which may improve patient management and reduce the cost of care.

1. Abbreviations

CIN: cervical intraepithelial neoplasia
DTS: Direct Tube Sampling
HC2: Hybrid Capture 2 HPV DNA
HPV: human papillomavirus
HR: high-risk
LA: Linear Array
mRNA: messenger RNA
NPV: negative predictive value
Pap: Papanicolaou
PCR: polymerase chain reaction
PPV: positive predictive value

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2. Background

Human papillomavirus (HPV) is a common sexually transmitted virus that infects most sexually active men and women sometime during their lifetime. Most sexually transmitted HPV infections are asymptomatic, do not cause disease, and are eventually cleared. However, a small minority of women will develop a persistent cervical infection, putting them at greater risk of developing cervical disease, including cervical cancer.[1,2] At least 14 HPV types have been shown to cause cervical cancer and are termed "high-risk" types.[3–6] These types are associated with 99.7% of all cervical cancers.[1]

Molecular diagnostic tests for HPV DNA have been incorporated into Papanicolaou (Pap) test screening programs in the United States and many other countries.[7] Screening programs based only on Pap testing have limitations, primarily due to sampling problems and the subjective interpretation

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of cytology results. HPV DNA testing is substantially more sensitive at detecting cervical disease (cervical intraepithelial neoplasia, CIN 2+) than cytology (96.1% vs. 53.0%), but is less specific (90.7% vs. 96.3%).[8] A combination of these two tests improves the detection of medium- and high-grade CIN and cervical cancer, because the sensitivity of the two tests combined is higher than that of either test alone.[8–11]

There are several HPV tests available designed to detect most or all of the high-risk HPV types. The Qiagen Hybrid Capture® 2 HPV DNA (HC2) Test and the Roche AMPLICOR® HPV Test (Amplicor) detect the DNA of 13 high-risk HPV types. The Roche Linear Array HPV Genotyping Test (LA) identifies 37 high- and low-risk HPV types. The Invader® HPV HR Molecular Assay (Invader) from Third Wave Technologies Inc. detects the DNA of 14 high-risk HPV types, as does the real-time PCR-based test from Abbott Molecular which also identifies women infected with HPV 16 and HPV 18. The Norchip Pretect Proofer (Proofer) test detects the E6/E7 messenger RNA (mRNA) of 5 high-risk HPV types. The Gen-Probe APTIMA® HPV Assay detects HPV E6/E7 mRNA from 14 high-risk HPV types.

Many studies have been published evaluating the performance of the HC2 test in comparison with histology. These studies have shown that the HC2 test sensitivity ranges from 85% to 100% for the detection of high-grade cervical disease or cancer.[9,12] The negative predictive value (NPV) was 99% to 100% when HC2 and Pap test results were combined, indicating the risk of developing high-grade cervical disease or cancer is very low in women with negative HPV DNA and Pap test results.[12] This very high NPV allows for longer screening intervals for women who are high-risk HPV negative and who have normal Pap test results. Accordingly, the American Society for Colposcopy and Cervical Pathology in conjunction with various participating organizations recommends a three-year screening interval for women if cervical cytology and HPV DNA tests are negative.[7] However, the HC2 test cross-reacts with some low-risk HPV types,[13,14] and has relatively low clinical specificity.[9]

Other studies have compared the clinical performance of the various HPV DNA tests. The Amplicor and HC2 tests have demonstrated similar sensitivity and specificity for the detection of high-risk HPV types,[15–17] as well as for the detection of cervical pre-cancer and cancer.[15,18–21] The LA test has been reported in some studies as having higher sensitivity, but lower specificity for detecting high-risk HPV among women with high-grade disease as compared to HC2.[19,22,23] The Invader test has been shown to have similar sensitivity and slightly better specificity as compared to the HC2 test.[24–26]

Most women with high-risk HPV DNA-positive results and normal Pap test results have transient HPV infections (80%) that will not progress to high-grade cervical disease or cancer.[27,28] These high-risk HPV DNA-positive results are false positives in relation to cervical disease, and decrease the clinical specificity and positive predictive value (PPV) of the DNA tests. The challenge becomes how to discriminate between transient and persistent infections, thereby improving the specificity of HPV testing. Nucleic acid amplification tests for the detection of E6/E7 mRNA have shown promise for increasing the specificity of HPV testing.[29,30] Expression of E6/E7 HPV mRNA is essential to the development and progression of cervical disease.[31] Detection of these mRNA targets could possibly result in a test that is more specific than DNA detection and able to predict which women will progress toward high-grade cervical disease.[32,33] Both the Proofer test and the APTIMA HPV Assay target HPV E6/E7 mRNA rather than DNA.

Several studies have compared the Proofer test with HC2 or PCR-based HPV DNA tests.[30,34] These studies have shown the Proofer test to have a lower clinical sensitivity for detection of cervical disease than the DNA tests, but a higher clinical specificity. The lower sensitivity and higher specificity of the Proofer test is due to the test's detection of 5 HPV types, rather than the 13 to 14 types detected by the DNA-based tests; the Proofer test's detection of mRNA rather than DNA may also contribute to its higher specificity.

The APTIMA HPV Assay was developed with the goal of combining the high specificity of an HPV mRNA test with the high sensitivity of an HPV DNA test by detecting the mRNA of 14 high-risk HPV types. Preliminary results with the APTIMA HPV Assay were first reported by Castle et al. for a cross-sectional study of 531 women undergoing testing for cervical disease.[35] APTIMA HPV Assay as well as LA and HC2 test results were compared with histology findings. There was a statistically significant correlation between the presence of HPV E6/E7 mRNA and progression toward cervical cancer. Fewer specimens tested positive for HPV E6/E7 mRNA than for HPV DNA, especially in women with histology results <CIN 1 or when no biopsy was taken, indicating detection of high-risk HPV mRNA may achieve similar clinical sensitivity and possibly better clinical specificity than the detection of high-risk HPV DNA.[35]

Although many reports have evaluated the clinical sensitivity and specificity of the HC2, Amplicor, LA and Proofer tests, only one publication has compared the performance of all four assays together with the APTIMA HPV Assay.[36] This study compared the sensitivity and specificity of multiple tests for the detection of high-grade CIN in 953 women referred to colposcopy because of abnormal cytology results. The sensitivity and specificity of the tests were determined on the basis of the most severe histology result (biopsy or treatment specimen). While the Proofer test had significantly higher specificity (73.1%) as compared to the other tests, it also had significantly lower sensitivity (73.6%). The APTIMA HPV Assay yielded similar sensitivity compared with the HC2, Amplicor and LA tests (95.2% vs. 99.6%, 98.9% and 98.2%) for the detection of CIN 2+, and had a significantly higher specificity (42.2% vs. 28.4%, 21.7% and 32.8%). The authors concluded that the HC2, Amplicor, LA, and APTIMA HPV tests showed very high sensitivity, indicating that they are unlikely to miss any significant disease, and of these tests, the APTIMA HPV Assay had the best specificity.[36]

The present study evaluated the clinical performance of the APTIMA HPV Assay for the detection of high-risk HPV and disease (CIN 2+ and CIN 3+) in clinical specimens in comparison with the HC2 test.

3. Materials and methods

3.1. Clinical specimens

A total of 800 residual PreservCyt liquid Pap specimens, collected from consenting French patients (Paris and Paris area) who were referred to colposcopy for follow-up or HPV associated disease were evaluated. Colposcopic evaluation was considered satisfactory if the squamo-columnar junction was clearly visible. Biopsies were obtained from those subjects with visible lesions at the time of colposcopy for which the histology was evaluated. Those subjects with a satisfactory colposcopy

and no visible lesion were not biopsied and were considered disease negative.

3.2. APTIMA HPV Assay

The APTIMA HPV Assay is a qualitative nucleic acid amplification test that detects HPV E6/E7 mRNA from 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) from liquid Pap specimens, but does not differentiate or identify the HPV type.[37] The APTIMA HPV Assay is designed to run on two instrument systems. One is the semi-automated Direct Tube Sampling Systems (DTS systems), which consists of a TECAN EVO pipettor (optional), a GEN-PROBE SB100 dry heat block and vortexer, a Target Capture system and a LEADER HC+ luminometer. The second system is the fully-automated TIGRIS DTS system (Gen-Probe Incorporated, San Diego, CA). One milliliter of each residual liquid Pap specimen was transferred to a buffered detergent solution (2.9 mL). A 400 microliter aliquot of the diluted liquid Pap specimen was then tested on the semi-automated DTS systems (n = 800), as well as on the fully-automated TIGRIS DTS system (n = 799) according to the instructions for use for the APTIMA HPV Assay.[37]

3.3. HC2 test

The HC2 test is a nucleic acid probe test for the qualitative detection of HPV DNA from 13 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) from clinical specimens. Residual liquid Pap specimens (n = 781) were tested according to the instructions for use.[14]

3.4. Linear Array test

The LA test is a qualitative test for the identification of 37 high- and low-risk HPV types in clinical specimens. Samples with discordant results between the APTIMA HPV Assay (either from DTS or TIGRIS DTS systems) and HC2 test (n = 137) were tested with the LA test according to the instructions for use[38] to resolve HPV status. A subset of samples with concordant APTIMA HPV Assay and HC2 test results (n = 256) were also tested with the LA test.

3.5. Detection of high-risk HPV

The sensitivity, specificity, PPV, and NPV of the APTIMA HPV Assay and HC2 test for the detection of high-risk HPV were determined by comparing the assay results with the high-risk HPV status of the specimens (n = 779). The high-risk HPV status was determined by concordance between the APTIMA HPV Assay and HC2 test. High-risk HPV status for those specimens with discordant results in the two assays was determined by the result from the LA test. Confidence intervals were calculated using the Score method.[39]

3.6. Detection of CIN

The clinical sensitivity and specificity of the APTIMA HPV Assay and HC2 test for the detection of cervical disease was determined by comparing the assay results with the disease status, as determined by colposcopy and histology results. Calculations were performed using both CIN 2+ and CIN 3+ as disease endpoints (n = 753). The Score method was used to calculate the 95% confidence intervals and the McNemar chi-square test to calculate p-values.[39,40]

4. Results

4.1. Detection of high-risk HPV

Table 1 presents the APTIMA HPV Assay results on both the semi-automated DTS and fully automated TIGRIS DTS systems, as well as the HC2 test results, in comparison with the high-risk HPV status (high-risk status was determined as described in section 3.5). Overall, 443 out of 779 specimens were high-risk HPV-positive and 336 were high-risk HPV-negative.

Of the 443 high-risk HPV-positive specimens, 407 (91.9%) were positive and 36 (8.1%) were negative in the APTIMA HPV Assay when tested on the semi-automated DTS systems. Of the 36 APTIMA HPV Assay negative specimens, 33 had no observed lesion or no high-grade lesions (<CIN 2), 2 were missing colposcopy/biopsy information and 1 had a CIN 2 result.

Similar APTIMA HPV Assay performance was observed on the TIGRIS DTS system: 410 (92.6%) were positive and 33 (7.4%) were negative. Of the 33 APTIMA HPV Assay negative samples, 29 were from women who had no observed lesion or no high-grade lesions (<CIN 2), 2 were missing colposcopy/biopsy information and 1 had a CIN 2 result.

Of the 443 high-risk HPV-positive specimens, 412 (93.0%) were positive in the HC2 test and 31 (7.0%) were negative. Of the 31 HC2 test negative samples, 27 were from women who had no observed lesion or no high-grade lesions (<CIN 2), 2 were missing colposcopy/biopsy information, 1 had a CIN 2 result and 1 had a CIN 3 result.

Of the 336 high-risk HPV-negative specimens, 331 (98.5%) were negative and 5 (1.5%) were positive in the APTIMA HPV Assay on the semi-automated DTS systems. Of these 5, three had no detectable HPV DNA by the LA test and were negative in the HC2 test. One of the remaining 2 was identified as HPV 53 and 62 positive, and 1 was identified as HPV CP6108 positive. Both of these specimens were also positive in the HC2 test.

Similar results were observed with the APTIMA HPV Assay on the TIGRIS DTS system: 330 (98.2%) specimens were negative and 6 (1.8%) were positive. Only one of the six positive samples on the TIGRIS DTS system was also positive on the semi-automated DTS systems but had no detectable HPV DNA by the LA test and was negative in the HC2 test. Two others also had no detectable HPV DNA by the LA test and were negative

Table 1
Comparison of APTIMA HPV ASSAY and HC2 test results with high-risk HPV status

	High-risk status		Total
	Positive	Negative	
APTIMA HPV DTS systems			
Positive	407	5	412
Negative	36	331	367
Total	443	336	779
APTIMA HPV TIGRIS DTS system			
Positive	410	6	416
Negative	33	330	363
Total	443	336	779
HC2			
Positive	412	61	473
Negative	31	275	306
Total	443	336	779

Table 2
Analytical sensitivity and specificity for detection of high-risk HPV (n = 779)

Parameter	APTIMA HPV Assay		HC2 test
	DTS systems	TIGRIS DTS system	
Sensitivity (95% CI)	91.9 (88.9–94.1)	92.6 (89.7–94.7)	93.0 (90.2–95.0)
p-value*	0.5287	0.7963	
Specificity (95% CI)	98.5 (96.6–99.4)	98.6 (96.9–99.3)	81.8 (77.4–85.6)
p-value*	<0.0001	<0.0001	
PPV	98.8	98.6	87.1
NPV	90.2	90.9	89.9

*McNemar Chi square test results when comparing APTIMA HPV Assay results to HC2 test.

in the HC2 test. Two of the remaining three specimens were identified as HPV 6, while the last one was identified as HPV 62 and 67. The three specimens that had low-risk types were also positive in the HC2 test.

Of 336 high-risk HPV negative specimens, 61 (18.2%) were positive in the HC2 test and 275 (81.8%) were negative. Of the 61 HC2 positive specimens, 15 had no detectable HPV DNA by the Linear Array test, and the other 46 had 1 or more of the following HPV DNA low-risk types identified: 6, 11, 42, 53, 54, 61, 62, 67, 70, 71, 72, 73 and CP6108. Only 5 of the 61 HC2 test positive specimens were positive in the APTIMA HPV Assay when tested on either the semi-automated DTS or fully-automated TIGRIS DTS systems.

Table 2 shows the analytical sensitivity, specificity, PPV, and NPV of the APTIMA HPV Assay (on both the semi-automated DTS and fully-automated TIGRIS DTS systems) and the HC2 test for detection of high-risk HPV. The sensitivity of the APTIMA HPV Assay on the DTS systems was 91.9%, the specificity was 98.5%, the PPV was 98.8%, and the NPV was 90.2%. The APTIMA HPV Assay sensitivity on the TIGRIS DTS system was 92.6%, the specificity was 98.2%, the PPV was 98.6%, and the NPV was 90.9%. HC2 test sensitivity was 93.0%, the specificity was 81.8%, the PPV was 87.1%, and the NPV was 89.9%. Sensitivity of the APTIMA HPV Assay was equivalent to that of the HC2 test (p-values >0.05). Specificity of the APTIMA HPV Assay was significantly higher than that of the HC2 test based on the 95% confidence intervals and p-values (<0.0001).

4.2. Detection of high-grade cervical lesions – CIN 2+

Summaries of the APTIMA HPV Assay and HC2 test results versus patient disease status are presented in Tables 3 and 4 for CIN 2+, and Tables 5 and 6 for CIN 3+. Of the 753 specimens with colposcopy or biopsy results, 141 (18.7%) were CIN 2+, whereas 87 (11.6%) were CIN 3+.

Of the 141 specimens with CIN 2+ results, 128 (90.8%) were positive in the APTIMA HPV Assay and 13 (9.2%) were negative on the semi-automated DTS systems (Table 3). Twelve of the 13 APTIMA HPV Assay-negative samples were also negative on the TIGRIS DTS system. The other specimen was positive on the TIGRIS DTS system, positive in the HC2 test, and high-risk HPV-positive in the LA test. Of the 612 specimens that were <CIN 2, 344 (56.2%) were negative in the APTIMA HPV Assay on the semi-automated DTS systems and 268 (43.8%) were positive. For the semi-automated DTS systems, the sensitivity of the APTIMA HPV Assay was 90.8%, the specificity was 56.2%, the PPV was 32.3%, and the NPV was 96.4% for detection of CIN 2+ (Table 4).

Table 3
Comparison of APTIMA HPV Assay and HC2 test results with histology results of CIN 2+

	CIN 2+		Total
	Positive	Negative	
APTIMA HPV DTS systems			
Positive	128	268	396
Negative	13	344	357
Total	141	612	753
APTIMA HPV TIGRIS DTS system			
Positive	128	273	401
Negative	13	339	352
Total	141	612	753
HC2			
Positive	134	322	456
Negative	7	290	297
Total	141	612	753

Table 4
Clinical sensitivity and specificity for detection of CIN 2+ (n = 753)

Parameter	APTIMA HPV Assay		HC2 test
	DTS systems	TIGRIS DTS system	
Sensitivity (95% CI)	90.8% (84.9–94.5)	90.8% (84.9–94.5)	95.0% (90.1–97.6)
p-value*	0.0578	0.0578	
Specificity (95% CI)	56.2% (52.3–60.1)	55.4% (51.4–59.3)	47.4% (43.5–51.4)
p-value*	<0.0001	<0.0001	
PPV	32.3%	31.9%	29.4
NPV	96.4%	96.3%	97.6

*McNemar Chi square test results when comparing APTIMA HPV Assay results to HC2 test.

Table 3 also presents the APTIMA HPV Assay results in the fully-automated TIGRIS DTS system versus disease status (CIN 2+). Of the 141 CIN 2+ specimens, 128 (90.8%) were positive in the APTIMA HPV Assay and 13 (9.2%) were negative. Twelve of the 13 negative specimens were also negative on the semi-automated DTS systems. Four of the 13 had no detectable HPV DNA by the LA test, 4 had low-risk HPV DNA identified (types 6, 6, 42 and 62), 4 were not tested in the LA test and 1 had HPV types 16, 52 and 84 identified. Of the 612 specimens that were <CIN 2, 339 (55.5%) were negative in the APTIMA HPV Assay on the TIGRIS DTS system and 273 (44.6%) were positive. Thus, for the TIGRIS DTS system, the sensitivity of the APTIMA HPV Assay was 90.8%, the specificity was 55.4%, the PPV was 31.9%, and the NPV was 96.3% for detection of CIN 2+ (Table 4).

The HC2 test results for the same specimens are also described in Table 3. Of the 141 CIN 2+ specimens, 134 (95.0%) were positive in the HC2 test and 7 (5.0%) were negative. Five of the 7 specimens were also negative in the APTIMA HPV Assay on both instrument systems; 1 had no detectable HPV DNA by the LA test and the other 4 were not tested in the LA test. The remaining 2 were positive in the APTIMA HPV Assay on both instrument systems and had high-risk HPV DNA detected by the LA test. Of the 612 specimens that were <CIN 2, 290 (47.4%) were negative in the HC2 test and 322 (52.6%) were positive. Thus, the sensitivity of the HC2 test was 95.0%, the specificity was 47.4%, the PPV was 29.4%, and the NPV was 97.6% for detection of CIN 2+ (Table 4).

Sensitivity of the APTIMA HPV Assay (on TIGRIS DTS and the semi-automated DTS systems) was equivalent to that of the HC2 test as shown by the overlapping confidence intervals and the p-values (>0.05). Specificity of the APTIMA HPV Assay (on both instrument systems) was statistically higher than that of the HC2 test based on the 95% confidence intervals and p-values (<0.001).

4.3. Detection of high-grade cervical lesions-CIN 3+

Similar analysis was performed using a clinical endpoint of CIN 3+ for the APTIMA HPV Assay on the semi-automated DTS and fully-automated TIGRIS DTS systems, as well as the HC2 test.

Of the 87 CIN 3+ specimens, 85 (97.7%) were positive in the APTIMA HPV Assay and 2 (2.3%) were negative on both instrument systems (Table 5). The 2 APTIMA HPV Assay-negative/CIN 3+ specimens were positive in the HC2 test, but had no detectable HPV DNA by the LA test. Of the 666 specimens that were <CIN 3, 355 (53.3%) were negative in the APTIMA HPV Assay on the semi-automated DTS systems and 311 (46.7%) were positive. On the TIGRIS DTS system, 350 (52.6%) were negative and 316 (47.4%) were positive. Thus, for the semi-automated DTS systems, the sensitivity of the APTIMA HPV Assay was 97.7%, the specificity was 53.3%, the PPV was 21.5%, and the NPV was 99.4% for detection of CIN 3+; and for the TIGRIS DTS system, the sensitivity of the assay was 97.7%, the specificity was 52.6%, the PPV was 21.2%, and the NPV was 99.4% for disease detection (Table 6).

HC2 test results for the same specimens are also described in Table 5. Of the 87 CIN 3+ specimens, 86 (98.9%) were positive in the HC2 test and 1 (1.1%) was negative. The HC2 test-negative specimen was positive in the APTIMA HPV Assay on both instrument systems and was identified as positive for HPV 16 and HPV 45 in the LA test. Of the 666 specimens that were <CIN 3, 296 (44.4%) were negative in the HC2 test and 370 (55.6%) were positive. Thus, the sensitivity of the HC2 test was 98.9%, the specificity was 44.4%, the PPV was 18.9%, and the NPV was 99.7% for detection of CIN 3+ (Table 6).

Sensitivity and specificity comparisons between the APTIMA HPV Assay and the HC2 test for CIN 3+ detection were similar to that seen for CIN 2+. Sensitivities of the two tests were similar; however, the specificity of the APTIMA HPV Assay was significantly higher than that of the HC2 test, based on the 95% confidence intervals and p-values.

Table 5
Comparison of APTIMA HPV Assay and HC2 test results with histology results of CIN 3+

	CIN 3+		Total
	Positive	Negative	
APTIMA HPV DTS systems			
Positive	85	311	396
Negative	2	355	357
Total	87	666	753
APTIMA HPV TIGRIS DTS system			
Positive	85	316	401
Negative	2	350	352
Total	87	666	753
HC2			
Positive	86	370	456
Negative	1	296	297
Total	87	666	753

Table 6
Clinical sensitivity and specificity for detection of CIN 3+ (n = 753)

Parameter	APTIMA HPV Assay		HC2 test
	DTS systems	TIGRIS DTS system	
Sensitivity (95% CI)	97.7% (92.0–99.4)	97.7% (92.0–99.4)	98.9% (93.8–99.8)
p-value*	0.5637	0.5637	
Specificity (95% CI)	53.3% (49.5–57.1)	52.6% (48.8–56.3)	44.4% (40.7–48.2)
p-value*	<0.0001	<0.0001	
PPV	21.5%	21.2%	18.9
NPV	99.4%	99.4%	99.7

*McNemar Chi square test results when comparing APTIMA HPV Assay results to HC2 test.

5. Discussion

5.1. Detection of high-risk HPV

The APTIMA HPV Assay is highly sensitive and specific for the detection of high-risk HPV in clinical specimens, with a sensitivity of 92% and a specificity of 98%. The results obtained in the DTS and TIGRIS DTS systems were equivalent. The HC2 test demonstrated similar sensitivity for detection of high-risk HPV (93%), but was less specific at 82%. The lower specificity of the HC2 test is likely due to the cross-reaction of HC2 test with low-risk subtypes.[13,14] In contrast, the APTIMA HPV Assay has not been reported to cross-react with low-risk subtypes.[37] Only 5 specimens were "false positive" in the APTIMA HPV Assay for presence of high-risk HPV on the semi-automated DTS systems and 6 specimens on the TIGRIS DTS system, compared to 61 specimens in the HC2 test. The false positive specimens in the APTIMA HPV Assay contained low-risk HPV types or no detectable HPV DNA according to LA test results. The specimens that contained low-risk types (6, 53, 62, 67 and CP6108) were all "false positive" in the HC2 test as well, which could be indicative of the presence of high-risk types that were missed by the LA test. Several studies have reported that the LA test does not identify all genotypes when more than one genotype is present due to primer competition.[41,42]

The APTIMA HPV Assay is designed to detect one more HPV type (HPV 66), than claimed by the HC2 test, that is considered high-risk in a number of publications.[31,43] For purposes of this study, those samples with discordant results between the APTIMA HPV Assay and the HC2 test that contained HPV 66 were considered high-risk HPV positive. Of the 10 specimens that were identified to contain only HPV 66, 9 were positive in the HC2 test indicating cross-reactivity with this type. While a high degree of cross-reactivity was observed with HPV 66 by the HC2 test, there were 7 specimens out of 26 that contained HPV 66 alone or with other HPV types that were negative in the HC2 test. Not detecting HPV 66 could lead to the failure of identifying women with an increased risk of developing cancer due to an infection with a high-risk HPV type.[31,43]

Overall, these results demonstrate that the APTIMA HPV Assay is as sensitive as the HC2 test but is significantly more specific than the HC2 test for the detection of high-risk HPV.

5.2. Detection of high-grade lesions

The APTIMA HPV Assay was highly sensitive and specific for detection of high-grade cervical lesions in the referral population tested in this study. The results were equivalent between the semi-automated DTS and fully-automated TIGRIS DTS systems. The APTIMA HPV Assay demonstrated similar sensitivity to the HC2 test for detection of CIN 2+ and CIN 3+, which is in agreement with previous reports.[35,36] Clinical specificity of APTIMA HPV Assay for detection of CIN 2+ and CIN 3+ was significantly higher than that of the HC2 test. The observed difference in specificity between the APTIMA HPV Assay and HC2 test translates into as many as 54 false positive specimens with the HC2 test in women with normal colposcopic findings or normal or CIN 1 histology when compared to the APTIMA HPV Assay results.

Biopsies were not taken from women when lesions were not visible upon satisfactory colposcopic exam; such patients were considered disease negative (<CIN 2). While it is possible that small lesions or lesions high up in the endocervical canal might not be detected, leading to patients incorrectly deemed to be disease negative, in a previous evaluation the sensitivity of colposcopy to recognize underlying high-grade CIN was greater than 95%.[18] Only subsequent follow-up of such patients would show the clinical value of a mRNA test compared to a DNA test.

The results of this study suggest that use of the APTIMA HPV Assay in cervical screening will detect most significant cervical disease while reducing false positive results, resulting in improved patient management and lower costs to the health care system.

Acknowledgements: We thank Liezel Morales for technical assistance. We thank Florence Paillard for support in writing the manuscript.

Competing interests: Janel Dockter, Astrid Schroder, Craig Hill, Leah Guzenski, Cristina Giachetti and Liezel Morales are employed by Gen-Probe Incorporated and hold stock options in that company. Joseph Monsonego has served on Advisory Boards for Merck, GSK, Qiagen, Roche, Abbott and GenProbe (honoraria and research funding).

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