Journal of Clinical Virology 45, S1 (2009) S49–S54

Contents lists available at ScienceDirect

Journal of Clinical Virology

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

Efficiency of the APTIMA® HPV Assay for detection of HPV RNA and DNA targets

Damon Getman[a,*], Aparna Aiyer[a], Janel Dockter[a], Cristina Giachetti[a], Fan Zhang[b], Christine C. Ginocchio[b]

[a]Gen-Probe Incorporated, San Diego, CA; [b]North Shore-LIJ Health System Laboratories, Department of Laboratory Medicine, Lake Success, NY, USA

ARTICLE INFO

Keywords:
HPV
Cervical cancer
RNA testing
Molecular diagnostics
APTIMA®
Cytology

ABSTRACT

Background: The APTIMA® HPV Assay (AHPV) is designed to detect HPV E6/E7 mRNA from 14 high-risk types in Cytyc PreservCyt liquid-based cytology specimens.
Objectives: To compare AHPV analytical sensitivity for RNA and DNA; to compare the sensitivity of AHPV and Hybrid Capture® 2 (HC2) assays for HPV DNA detection; to compare assay performance with and without sample denaturation; to compare assay results with cytology.
Study design: Analytical sensitivity of AHPV for detecting E6/E7 RNA was assessed by spiking samples with various quantities of HPV 16 E6/E7 *in vitro* RNA transcript or HPV 16-positive SiHa cells. AHPV and HC2 analytical sensitivity for HPV 16 DNA was evaluated by spiking samples with various quantities of a plasmid vector containing cloned HPV 16 DNA, or with purified SiHa cell genomic DNA containing integrated HPV 16 genome. Samples were tested using standard AHPV and HC2 protocols. Endocervical samples from 568 women were collected in Digene Specimen Transport Medium. Non-denatured and denatured samples were tested in AHPV and denatured samples with HC2. Assay results were compared to each other, and to cytology.
Results: AHPV had substantially higher (2–4 $\log_{10}$) analytical sensitivity for HPV 16 RNA than for HPV 16 DNA. AHPV also had substantially lower (3 $\log_{10}$) analytical sensitivity for HPV 16 DNA compared to HC2. The overall agreement between assay results in clinical specimens was 94.2%, but AHPV had fewer positives than HC2 (48.4% positive agreement). In denatured samples, the number of samples testing positive in AHPV increased two-fold, yielding a positive agreement rate of 88.7%. When assay results were compared with cytology, AHPV had fewer positives in samples with normal or ASC-US diagnoses than did HC2.
Conclusions: AHPV is much more efficient at detecting HPV 16 RNA than DNA. Selective capture, amplification, and detection of HPV RNA by AHPV may improve the specificity of molecular HPV testing.

1. Introduction

The low sensitivity (<80%) of the Papanicolaou (Pap) smear for the detection of cervical disease has prompted the development of new, highly sensitive, amplified nucleic acid tests for the detection of HPV in clinical samples. These new tests are based on the detection of the nucleic acids from high-risk (HR) HPV genotypes in cervical samples. The Digene Hybrid Capture® 2 HPV DNA Test (HC2) (Qiagen, Gaithersburg, MD), the Roche AMPLICOR® HPV Test (Roche Molecular Systems, Roche Diagnostics, Mannheim, Germany), and the Abbott RealTi*me* High Risk HPV assay (Abbott Laboratories, East Windsor, NJ) are designed to detect the presence of the *L1* gene in viral genomic DNA from 13 to 14 HR HPV genotypes, while the Norchip PreTect Proofer test (Norchip, Klokkarstua, Norway) detects E6/E7 oncogenic RNA from 5 HR HPV genotypes. Tests based on HPV DNA detection have been shown to have lower specificity for histopathologically-confirmed disease compared to tests which detect HPV RNA,[1] probably due to the fact that most cervical HPV infections have relatively high amounts of HPV DNA but low amounts of E6/E7 mRNA and do not result in neoplasia or cancer. Indeed, many studies comparing

* Corresponding author. Damon Getman, Ph.D. Gen-Probe Incorporated, 10210 Genetic Center Dr, San Diego, CA 92121, USA. Tel.: +1 (858) 410 8537; fax: +1 (858) 410 8871.
E-mail address: damong@gen-probe.com (D. Getman).

1590-8658/ $ – see front matter

assay performance with histology results have shown that the specificity of HC2 and AMPLICOR HPV for the detection of grade 2 or greater cervical intraepithelial neoplasia (CIN 2+) is relatively low, ranging between 22% and 65%.[2–7] The low specificity of these assays implies that a large number of women may be unnecessarily referred to colposcopy with biopsy. Thus, there is an important need for a highly sensitive test for HR HPV with improved specificity.

Gen-Probe Incorporated has recently produced a new CE-marked HPV test, the APTIMA® HPV Assay (AHPV), which is designed to detect oncogenic E6/E7 mRNA from 14 high-risk HPV (HR HPV) genotypes, rather than viral genomic DNA from HR HPV genotypes. The utility of the AHPV test is derived from the fact that persistent infections with HR HPV types may result in virus integration into the host cell genome, leading to the overexpression of E6/E7 mRNA.[8] Viral E6 and E7 proteins bind to cellular p53 and pRB proteins, disrupting their regulatory functions. The ensuing unregulated host cell proliferation ultimately leads to neoplastic cellular transformation.[9]

The AHPV assay procedure involves cell lysis and capture of the target E6/E7 HPV mRNA, directly amplifying the target RNA using transcription-mediated amplification (TMA), and then detecting the resulting RNA amplicons using chemiluminescent probes. To detect HR HPV RNA but not DNA, the assay target capture step is optimized to capture only single-stranded nucleic acids. Unlike the HC2, Amplicor, and Abbott RealTi*m*e assays, which use thermal or chemical methods to denature HPV double-stranded DNA, the isothermal TMA method used in AHPV favors amplification of single-stranded nucleic acids. Any residual viral genomic DNA that eludes the oligonucleotide-based target capture system remains double-stranded during the subsequent isothermal amplification reaction and thus serves as a poor substrate for primer extension. Combined, these methods result in an assay that is much more sensitive for detecting viral RNA targets than viral DNA targets.

This study was conducted to evaluate the performance of the AHPV assay for detecting HPV E6/E7 mRNA versus HPV DNA. For this, we first determined the analytical sensitivity of AHPV for the detection of HPV RNA and DNA. The analytical sensitivity of AHPV for HPV DNA detection was also compared to that of HC2. Second, we compared the performance of AHPV and HC2 assays in clinical specimens with and without sample denaturation. Third, we compared AHPV and HC2 assay results in clinical specimens to cytology results.

2. Material and methods

2.1. AHPV assay

The AHPV assay is an amplified nucleic acid test that allows for qualitative detection of 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) in cervical specimens.[9] The assay is validated for Pap smear specimens collected in ThinPrep vials containing PreservCyt transport solution (Cytyc Corporation, Boxborough, MA, USA), and for specimens collected with the APTIMA Cervical Specimen Collection and Transport Kit (Gen-Probe Incorporated, San Diego, CA, USA). AHPV involves three main steps, which take place in a single tube: first, the target mRNA is captured using target-specific oligomers linked to magnetic microparticles; second, the isolated target mRNA is amplified using TMA; third, the amplification products are detected using chemiluminescent-labeled probes in the Hybridization Protection Assay (HPA), with hybridized probe signals measured in relative light units (RLU). An internal control, added to all samples at the target capture step, mitigates the risk of false-negative results. Positive and negative calibrators are used to determine the cut-off values for the internal control and analyte signals in each run. All samples in the current study were tested in accordance with the manufacturer's instructions.

2.2. HC2 assay

HC2 is an *in vitro* 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. The assay utilizes antibody capture of HPV DNA–RNA probe hybrids and qualitative chemiluminescent signal detection based on signal amplification. All samples in the current study were tested according to the manufacturer's package insert.

2.3. Analytical sensitivity of AHPV for the detection of E6/E7 HPV mRNA

To determine the analytical sensitivity of the AHPV assay for HPV RNA, either *in vitro* transcribed (IVT) RNAs corresponding to full-length HPV 16 E6/E7 mRNA or intact HPV 16-positive SiHa cells were spiked into a negative sample matrix consisting of a 1:3 mixture of pooled AHPV-negative PreservCyt liquid-based cytology (LBC) clinical specimens and APTIMA specimen transport medium (STM). SiHa cells were obtained from ATCC (Cat. No. HTB-35, ATCC, Manassas, VA, USA). IVT RNA was spiked at concentrations corresponding to 1×10^1, 3×10^1, 1×10^2, 3×10^2, 1×10^3, and 3×10^3 copies/mL. SiHa cells were spiked at 0.1, 0.3, 1, 3, 1×10^1, and 3×10^1 cell equivalents/mL. These samples were then tested in the AHPV assay on the fully automated TIGRIS DTS System.

2.4. Analytical sensitivity of AHPV and HC2 for the detection of HPV DNA

Uncut circular pBlueScript plasmid vectors containing cloned HPV 16 gene sequences (derived from episomal double-stranded HPV 16 DNA) and genomic DNA extracted from SiHa cells (containing 1–2 copies of HPV 16 genome integrated per cell genome) were used to model episomal and integrated HPV genomic DNAs, respectively. HPV 16 plasmid DNAs were prepared using QIAGEN Plasmid Midi Kit (Qiagen, Valencia, Calif). Genomic DNA from SiHa cells was prepared using the QIAGEN DNeasy Kit (Qiagen). RNase was used during the extraction to remove HPV 16 RNA from the sample.

To determine the analytical sensitivity of AHPV for HPV DNA, HPV 16 E6/E7 plasmid DNA or purified SiHa genomic DNA were spiked into a negative sample matrix composed of a 1:3 mixture of pooled AHPV-negative PreservCyt LBC samples and APTIMA STM. HPV 16 E6/E7 plasmid DNA was spiked at concentrations corresponding to: 2×10^2, 6×10^2, 2×10^3, 6×10^3, 2×10^4, 6×10^4, 2×10^5, 6×10^5, 2×10^6, 6×10^6, and 2×10^7 copies/mL. Since SiHa cells are aneuploid (~ triploid),[10] 10 pg of SiHa genomic DNA was considered equivalent to one SiHa cell. It was assumed that one SiHa cell contained, on average, 1.5 copies of integrated HPV 16.[10] SiHa genomic DNA was spiked at concentrations corresponding to 1×10^2, 3×10^2, 1×10^3, 3×10^3, 1×10^4, 3×10^4,

Table 1
Analytical sensitivity of APTIMA® HPV Assay for HPV RNA

		% of positive samples
HPV 16 E6/E7 RNA *in vitro* transcript (copies/mL)		**N = 60**
0		0%
10		1.7%
30		23.3%
100		43.3%
300		93.3%
1,000		100%
3,000		100%
SiHa cells (number of cells/mL)	**HPV 16 E6/E7 RNA** (estimated copies/mL[a])	**N = 60**
0	0	0%
0.1	50	8.3%
0.3	150	15.0%
1	500	23.3%
3	1,500	73.3%
10	5,000	98.3%
30	15,000	100%

[a] Assumes each SiHa cell contains approximately 500 copies/cell HPV 16 E6/E7 RNA.[10]

1×10^5, 3×10^5, and 1×10^6 SiHa cell equivalents/mL. These samples were tested in AHPV following the package insert instructions.

For HC2 testing, uncut plasmid containing whole genomic HPV 16 DNA was spiked at concentrations corresponding to 2×10^2, 6×10^2, 2×10^3, 6×10^3, 2×10^4, 6×10^4, 2×10^5, 6×10^5 and 2×10^6 copies/mL. SiHa genomic DNA was spiked at 1×10^2, 3×10^2, 1×10^3, 3×10^3, 1×10^4, and 3×10^4 SiHa cell equivalents/mL. These samples were tested at TriCore Reference Laboratories (Albuquerque, NM, USA) using the HC2 assay.

2.5. Clinical performance of AHPV and HC2 before and after sample denaturation

To compare the clinical performance of the AHPV and HC2 assays, endocervical samples from 568 women were collected in Digene STM-based transport tubes. The majority of the Digene STM samples (n = 512) were from women ⩾30 yr, who had HPV testing performed as part of routine screening in conjunction with a liquid-based Pap. To avoid the formation of precipitates, an aliquot was removed from each Digene STM-based sample, diluted 1/15 in phosphate-buffered saline and then 1/4 into the APTIMA STM, and tested by AHPV. A denaturing agent containing NaOH was then added to the remaining sample in Digene STM, and tested subsequently by HC2 and AHPV. The agreement between AHPV assay results (before and after sample denaturation) and the standard HC2 assay result (using denatured samples only) was determined for all patients. The same analysis was also performed for a subset of 460 patients (women ⩾30 years of age who had a Pap result of Within Normal Limits [WNL]). For each patient, results from both assays were also compared to available cytology (Pap) results. Cytology results were classified as WNL, ASC-US (atypical squamous cells of undetermined significance), LSIL (low-grade squamous intraepithelial lesion), and HSIL (high-grade squamous intraepithelial lesion).

3. Results

3.1. Analytical sensitivity of AHPV for HPV RNA and DNA

Using HPV 16 E6/E7 IVT RNA diluted in an HPV-negative sample matrix, the AHPV assay detected 1000 copies/mL of HPV 16 RNA (equivalent to 100 copies of HPV 16 E6/E7 RNA per reaction) with 100% positivity; the 95% detection level determined by Probit analysis was 452 copies/mL (95% fiducial limits: 247–1501, Table 1). Using HPV 16 E6/E7 RNA-containing SiHa cells diluted in an HPV-negative sample matrix, the AHPV assay detected 30 cells/mL of sample (equivalent to three cells per reaction, or 15,000 copies/mL of HPV 16 E6/E7 RNA) with 100% positivity; the 95% detection level determined by Probit analysis was 6032 copies/mL (95% fiducial limits: 2268–77,787, Table 1). The 100% detection level of the AHPV assay for purified HPV 16 plasmid DNA (representing episomal double-stranded HPV 16 DNA) was 2×10^7 copies/mL; the 95% detection level determined by Probit analysis was 9.54×10^6 copies/mL (95% fiducial limits: 5.49×10^6–2.85×10^7, Table 2). For SiHa cell-integrated HPV 16 DNA, the 100% detection level of the AHPV assay was 1.5×10^6 copies/mL; the 95% detection level determined by Probit analysis was 1.15×10^6 copies/mL (95% fiducial limits: 6.22×10^5–6.39×10^6, Table 2). Thus, AHPV was over 20,000 times more sensitive for the detection of HPV 16 E6/E7 RNA than for the detection of episomal HPV 16 DNA. Using HPV 16-positive SiHa cells as the target source of RNA or DNA, AHPV was approximately 190 times more sensitive for detection of intracellular HPV 16 E6/E7 RNA than for detection of integrated HPV 16 DNA. Thus, overall the analytical sensitivity of AHPV for HPV E6/E7 RNA was much higher (2–4 $\log_{10}$) than that for HPV DNA.

3.2. Comparison of the analytical sensitivity of AHPV and HC2 for HPV DNA

The 100% detection level of the HC2 assay was 6×10^3 copies/mL for episomal HPV 16 double-stranded DNA and 1.5×10^3

Table 2
Analytical sensitivity of APTIMA® HPV Assay and HC2® for HPV DNA

		APTIMA HPV Assay	HC2 (RLU/CO ratio) [a]
HPV 16 episomal DNA (HPV 16 purified plasmid copies/mL)		**N = 15**	**N = 10**
200		Neg	Neg (0.22)
600		Neg	Neg (0.47)
2000		Neg	Neg (0.75)
6000		Neg	100% Pos (1.91)
20,000		Neg	100% Pos (6.05)
60,000		Neg	100% Pos (16.93)
200,000		Neg	100% Pos (52.99)
600,000		13% Pos	100% Pos (146.83)
2,000,000		47% Pos	100% Pos (461.19)
6,000,000		87% Pos	ND
20,000,000		100% Pos	ND
SiHa purified genomic DNA (cell equivalents/mL) [b]	**Integrated HPV 16 DNA** (copies/mL)	**N = 10**	**N = 10**
0	0	Neg	Neg (0.2)
100	150	Neg	Neg (0.35)
300	450	Neg	Neg (0.66)
1000	1500	Neg	100% Pos (1.57)
3000	4500	Neg	100% Pos (4.20)
10,000	15,000	Neg	100% Pos (12.66)
30,000	45,000	Neg	100% Pos (35.23)
100,000	150,000	30% Pos	ND
300,000	450,000	60% Pos	ND
1,000,000	1,500,000	100% Pos	ND

[a] The average RLU/Cutoff ratio for each concentration of DNA tested in the Digene HC2 assay is shown in parentheses. ND, not done.
[b] Assumes each SiHa cell equivalent contains approximately 1.5 copies of integrated HPV 16 DNA.[10]

copies/mL for SiHa integrated HPV 16 DNA, while that for AHPV was 2×10^7 copies/mL and 1.5×10^6 copies/mL, respectively (Table 2). Thus, the HC2 assay was 1000 times (for cell genome-integrated HPV DNA) to over 3000 times (for episomal HPV DNA) more sensitive than AHPV for the detection of HPV DNA.

3.3. Comparison of AHPV and HC2 assay results in clinical specimens with or without sample denaturation

A total of 568 endocervical specimens were collected in Digene STM. Of these 568 specimens, 56 were from women <30 years of age and 512 were from women ⩾30 years. The AHPV results both before and after sample denaturation were compared to HC2 results obtained from denatured samples (Table 3). The denaturing agent contains NaOH, which degrades the RNA and denatures the double-stranded DNA. Without denaturation, the agreement between the assays for negative and overall results was high (99.8% and 94.2%, respectively), while the agreement for positive results was low (48.4%) due to the HC2 assay detecting twice as many positives (62 vs. 31 for AHPV, Table 3). With sample denaturation, however, positive agreement between assays increased to 88%, which was due solely to an increase in positive results in AHPV (from 31 to 69, Table 3).

Table 3
Comparison of AHPV results (with and without sample denaturation) with HC2® results (with sample denaturation) – all clinical specimens

AHPV assay results	HC2 assay results		
	Positive	Negative	Total
Non-denatured samples			
Positive	30	1	31
Negative	32	505	537
Total	62	506	568
Assay agreement: Positive, 48.4%; Negative, 99.8%; Overall, 94.2%			
Denatured samples			
Positive	55	14	69
Negative	7	492	499
Total	62	506	568
Assay agreement: Positive, 88.7%; Negative, 97.2%; Overall, 96.7%			

AHPV: APTIMA® HPV Assay.

3.4. Comparison of assay results with cytology

Overall, AHPV positive rates with non-denatured samples were consistently lower compared to AHPV positive rates with denatured samples, and lower compared to HC2 positive rates, regardless of the patient cytology status (Table 4). In contrast, AHPV positive rates in denatured samples were similar to HC2

Table 4
Correlation between AHPV or HC2 assay results and cytology

Assay	WNL (N = 498)	ASC-US (N = 21)	LSIL (N = 9)	HSIL (N = 4)
AHPV (non-denatured)	12 (2.4%)	8 (38.0%)	7 (77.8%)	3 (75.0%)
AHPV (denatured)	39 (7.8%)	11 (52.4%)	8 (88.9%)	4 (100%)
HC2	33 (6.6%)	12 (57.1%)	8 (88.9%)	4 (100%)

AHPV: APTIMA® HPV Assay.

positive rates in all cytology grade categories. Compared with AHPV results for non-denatured samples, HC2 yielded a higher rate of positive results in patients with WNL (n = 498) or ASC-US (n = 21) cytology: the rate of positive results for WNL patients was 6.6% in HC2 and 2.4% in AHPV; and for ASC-US patients, 52.4% in HC2 and 38.0% in AHPV. The prevalence of LSIL and HSIL in this population was 2.4% (13/532). The positive rates for samples with LSIL and HSIL cytology status were high in both assays (for LSIL, 88.9% in HC2 and 77.8% in AHPV; for HSIL, 100% in HC2 and 75.0% in AHPV).

4. Discussion

In this study, we found that AHPV has a substantially higher analytical sensitivity for HPV 16 E6/E7 RNA than for HPV 16 E6/E7 DNA. Moreover, compared to the HC2 assay, AHPV has a much lower analytical sensitivity for HPV DNA. This confirms that the AHPV assay preferentially detects HPV E6/E7 RNA. This target selectivity is likely due to the AHPV assay's target capture technology, which is optimized to capture single-stranded nucleic acids but not double-stranded DNA, and the TMA process used in AHPV, which is isothermal and does not promote denaturation of double-stranded DNA hybrids.

Interestingly, in this study AHPV showed approximately 8-fold higher 95% sensitivity level for detecting integrated HPV 16 DNA than for episomal form HPV 16 E6/E7 plasmid DNA. This sensitivity analysis is based on the published data showing that SiHa cells contain 1–2 copies of integrated HPV 16 per cell genome.[10] Earlier published data suggested that SiHa cells may contain up to 10 copies of HPV 16 genome per cell genome.[11] Use of this higher integrated genome copy number value for the AHPV assay sensitivity calculations of Table 2 yields a 95% reactive level of the AHPV assay for SiHa cell-based integrated HPV 16 DNA of 7.64×10^6 copies/mL, which is similar to the value of 9.54×10^6 copies/mL obtained for the 95% reactive rate of AHPV for episomal HPV 16 DNA. Thus the line of SiHa cells used in this study may contain a higher number of integrated HPV 16 genomes than assumed, which in turn would increase proportionally the relative sensitivity of the AHPV assay for cell-based HPV RNA compared to cell-based HPV DNA.

While negative agreement between the HC2 and AHPV assays is similar in denatured and non-denatured samples, assay agreement for positive detection increases substantially (from 48.4% to 88.4%) when AHPV is performed on denatured samples. This was due to a 2-fold increase in the number of positive results with AHPV after sample denaturation. This could be explained by the fact that the denaturing reagent separates the double-stranded HPV DNA into single-stranded nucleic acids, which can then be captured and amplified in the AHPV assay. Thus, patients whose cervical scrape samples that are AHPV-negative when tested in the native state, but turn positive by AHPV when denatured, may harbor productive HPV infections containing high levels of episomal viral DNA but relatively low levels of oncogenic E6/E7 RNA. Since cytological abnormalities in these patients may be more likely to regress as the HPV infection is cleared,[12] a lower detection rate obtained using HPV RNA molecular testing may correspond to improved clinical specificity compared to HPV DNA testing.

Our results also showed that the non-denatured Digene STM-based clinical samples, when diluted 1/60 as described in the *Materials and methods* section, may be an acceptable source for AHPV testing, since AHPV and HC2 overall agreement using Digene STM-based samples was high (94.2%). This implies that clinical specimens harvested in Digene STM for testing in HC2 can be reliably retested in AHPV, provided that the samples are stable when diluted as described.

Compared with HC2, AHPV yielded a significantly lower rate of positives (2.4% for AHPV vs. 6.6% for HC2; $P < 0.05$) in patients with WNL cytology. Similarly, for patients with ASC-US cytology, HC2 had a higher rate of positive results (57.1%) compared to AHPV (38.0%), though this difference was not significant. These results are in agreement with previously published results that compared the performance of HC2 and AHPV in clinical specimens.[1] In that study, assay sensitivity and specificity for the detection of high-grade CIN was determined on the basis of the worst histology in 953 women referred to colposcopy. Those results showed that AHPV had a similar sensitivity to HC2 (95.2% vs. 99.6%), but a significantly higher specificity (42.2% vs. 28.4%) than HC2 for the detection of moderate to high-grade CIN (CIN 2+).

A limitation of our study was the relatively small number of HPV-positive samples with cytology diagnosis available for use in evaluating the comparative performance of the AHPV and HC2 tests. While the HC2-based HPV prevalence in this cohort (majority $\geqslant$30 years of age) is consistent with that previously reported,[13] the opportunity to test a larger number of HPV-positive women with normal to moderately abnormal (ASC-US/LSIL) cytology diagnoses would improve the power of the analysis presented here and would provide a more accurate assessment of the potential improvement in clinical specificity of cervical cancer screening procedures afforded by including the detection of HPV oncogenic RNA.

Taken together, these results show that the selective targeting of HR HPV E6/E7 mRNA (instead of HR HPV DNA) reduces the positive detection rate of HPV infections in women with normal or ASC-US cytology diagnoses. Hence, the presence of HPV E6/E7 mRNA in clinical specimens may be a more accurate predictor of cervical cell dysplasia and transformation, while the presence of HPV DNA only denotes HPV infection, which is most often transient and cleared without further disease development.[12] The relatively lower specificity of HPV DNA-based assays implies that a large number of women may be unnecessarily referred to colposcopy with biopsy, a procedure that is invasive, painful, costly, and may produce adverse effects.

Acknowledgements: We thank Sunghae Joo for assistance with Probit analysis and Florence Paillard for her support in writing the manuscript.

Competing interests: Damon Getman, Aparna Aiyer, Janel Dockter and Cristina Giachetti are employees of Gen-Probe Incorporated and hold stock options in the company. Christine C. Ginocchio: Research funding and member of the Scientific Advisory Board (honoraria). Fan Zhang has nothing to declare.

References

1. Szarewski A, Ambroisine L, Cadman L, Austin J, Ho L, Terry G, et al. Comparison of predictors for high-grade cervical intraepithelial neoplasia in women with abnormal smears. *Cancer Epidemiol Biomarkers Prev* 2008;**17**:3033–42.
2. Monsonego J, Bohbot JM, Pollini G, Krawec C, Vincent C, Merignargues I, et al. Performance of the Roche AMPLICOR human papillomavirus (HPV) test in prediction of cervical intraepithelial neoplasia (CIN) in women with abnormal PAP smear. *Gynecol Oncol* 2005;**99**:160–8.
3. Sandri MT, Lentati P, Benini E, Dell'Orto P, Zorzino L, Carozzi FM, et al. 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* 2006;**44**:2141–6.
4. Carozzi F, Bisanzi S, Sani C, Zappa M, Cecchini S, Ciatto S, et al. 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* 2007;**45**:364–9.
5. De Francesco MA, Gargiulo F, Schreiber C, Ciravolo G, Salinaro F, Manca N. Comparison of the AMPLICOR human papillomavirus test and the hybrid capture 2 assay for detection of high-risk human papillomavirus in women with abnormal PAP smear. *J Virol Methods* 2008;**147**:10–7.
6. Stevens MP, Garland SM, Rudland E, Tan J, Quinn MA, Tabrizi SN. 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* 2007;**45**:2130–7.
7. Wahlström C, Iftner T, Dillner J, Dillner L; Swedescreen study group. Population-based study of screening test performance indices of three human papillomavirus DNA tests. *J Med Virol* 2007;**79**:1169–75.
8. Pett M, Coleman N. Integration of high-risk human papillomavirus: a key event in cervical carcinogenesis. *J Pathol* 2007;**212**:356–67.
9. Gen-Probe APTIMA HPV Assay [package insert]. Gen-Probe Inc., San Diego, CA, USA; 2008.
10. Baker C, Phelps W, Lindgren V, Braun M, Gonda M, Howley P. Structural and transcriptional analysis of human papillomavirus type 16 sequences in cervical carcinoma cell lines. *J Virol* 1987;**61**:962–71.
11. Yee C, Krishnan-Hewlett I, Baker C, Schlegel R, Howley P. Presence and expression of human papillomavirus sequences in human cervical cancer cell lines. *Am J Pathol* 1985;**119**:361–366.
12. Baseman JG, Koutsky LA. The epidemiology of human papillomavirus infections. *J Clin Virol* 2005;**32**:S16–24.
13. Cuzick J, Clavel C, Petry K, Meijer C, Hoyer H, Ratnam S, et al. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. *Int J Cancer* 2006;**119**:1095–1101.