Retrospective and prospective evaluation of the Amplicor HPV test for detection of 13 high-risk human papillomavirus genotypes on 862 clinical samples

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ABSTRACT

Background: Persistent infection with a subgroup of at least 15 high-risk human papillomavirus (HPV) genotypes is considered as a necessary although insufficient etiological factor in the development of cervical carcinoma. As a consequence, HPV testing has recently become an important part of the cervical carcinoma screening and detection algorithms.
Aim of the study: To evaluate the analytical performance of a recently developed polymerase chain reaction (PCR)-based Amplicor HPV test (Roche Molecular Systems) in comparison with the Hybrid Capture 2 HPV DNA test (hc2) (Digene Corporation) for the detection of 13 high-risk HPV genotypes. In-house consensus PGMY09/PGMY11 and CPI/IIg PCRs targeting two different HPV genes coupled with HPV genotyping were used as an HPV internal reference standard.
Materials and methods: In the retrospective evaluation, 550 cervical scrape specimens with previously established HPV status were included. Additionally, 312 cervical scrape specimens were tested prospectively for the presence of 13 high-risk HPV genotypes by both hc2 and Amplicor HPV test.
Results: In the retrospective evaluation, the Amplicor HPV test results agreed almost completely with the HPV internal reference standard results. In the prospective evaluation performed on 312 samples, the concordant Amplicor and hc2 results were obtained in 85.9% of samples tested.
Conclusion: In our hands, the Amplicor HPV test demonstrated high analytical sensitivity and specificity. The higher analytical specificity of Amplicor in comparison to that of hc2 can be considered clinically useful. Prospective studies with clinical endpoints are urgently needed to assess the clinical utility of the higher analytical sensitivity of the Amplicor HPV test for primary HPV screening and triaging patients with ASC-US.

KEY WORDS

human papillomavirus, HPV, Amplicor, hybride capture, PCR

Introduction

Human papillomaviruses (HPV) are the most common sexually transmitted microorganisms. Persistent infection with a subgroup of at least 15 high-risk human papillomavirus (HPV) genotypes is considered as a necessary although insufficient etiological factor in the development of cervical carcinoma (1-3). As a conse-

quence, HPV testing has become an important part of the cervical carcinoma screening and detection algorithms (2, 3). As a consequence of this the US Food and Drug Administration (FDA) has recently approved a concurrent HPV and Pap smear screening of women aged 30 years and more. Additionally, several consensus guidelines recommend HPV testing when evaluating patients with a cytological diagnosis of atypical squamous cells of undetermined significance (ASC-US) (4, 5).

Hybrid Capture 2 HPV DNA test (hc2) (Digene Corporation, Gaithersburg, MD), a microplate-based solution and solid phase hybridization assay for the detection of 13 high-risk (16/18/31/33/35/39/45/51/52/56/58/59/68) and 5 low-risk (6/11/42/43/44) HPVs, is at present the only FDA approved assay for the routine detection of HPV infections and the only commercially available HPV DNA assay with sufficient scientific data to support its performance in a clinical setting (2, 6, 7). Although hc2 is the most widely used HPV test and many studies have demonstrated that hc2 is a sensitive and reliable test for the detection of HPV (8-13), several recent studies showed a significant analytical inaccuracy of the hc2 test near to cut-off, mainly due to the cross-reactivity of its high-risk probe cocktail (14-20).

In mid-2004, the polymerase chain reaction (PCR)-based Amplicor HPV test (Roche Molecular Systems, Branchburg, NJ) was launched on the European market. The Amplicor HPV test is designed to detect the same 13 high-risk HPV genotypes as the hc2 high-risk probe cocktail and, in principle, has been developed to be used for high-risk HPV screening. To the best of our knowledge, only one evaluation of the Amplicor HPV test has been published to date, namely its comparison with the other PCR-based commercially available assay INNO-LiPA HPV test (Innogenetics, Gent, Belgium) (21). In addition, the performance of the Amplicor HPV test in prediction of cervical intraepithelial neoplasia in women with abnormal cervical smears using colposcopic biopsy and liquid-based cytology as the reference standards, has been published recently (22).

In the present study, we evaluated retrospectively and prospectively the analytical performance of the Amplicor HPV test in comparison to hc2 for their ability to detect reliably 13 high-risk HPV genotypes: HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV68 in the settings of a routine diagnostic laboratory. Since HPV analytical standards or proficiency testing samples are still neither established nor commercially available, a comparison of any two HPV diagnostic tests is problematic (2). Therefore, in such cases, one method (or combination of methods) should be appointed as reference standard. In our study, similarly to early evaluations of molecular methods for *Chlamydia trachomatis*, two highly sensitive and specific consensus PCRs targeting two different HPV genes (L1 and E1 genes) coupled with reliable genotyping methods served as internal reference HPV standard.

Materials and methods

Retrospective evaluation

In the retrospective evaluation, 550 cervical scrape specimens with previously resolved HPV status were included. These specimens originated from two sources. The first source was our study in which the specificity and accuracy of the hc2 high-risk probe cocktail was determined by exact genotyping of cervical samples obtained from 310 women recognized as HPV positive using the hc2 high-risk probe cocktail (16). The second source was a Slovenian hc2 borderline results prospective study in which 240 samples with repeatedly borderline/equivocal/indeterminate hc2 results (defined as specimens with the repeated relative light units/cut-off ratio hc2 values ranging between 0.4 and 4.0) were identified and their HPV status carefully evaluated (submitted for publication).

For the determination of HPV status, all 550 samples were previously tested using PGMY09/PGMY11 in-

Table 1. Results of the retrospective evaluation of the Amplicor HPV Test and Hybrid Capture 2 HPV DNA test (hc2) for the detection of 13 high-risk HPV genotypes on 550 cervical scrape specimens with previously resolved HPV status.

hc2 status*	**AMPLICOR**		
	HPV positive	*HPV negative*	*Total*
hc2 true positive samples	218	0	218
hc2 true negative samples	2	257	259
hc2 false negative samples	27	0	27
Hc2 false positive samples	2	44	46
Total	249	301	550

**as determined according to the internal reference HPV standard*

house HPV consensus PCR targeting app. 450 bp of L1 gene, as described previously (10, 16). To exclude HPV infection definitively, all PGMY09/PGMY11 PCR negative samples were additionally tested using CPI/IIg in-house HPV consensus PCR targeting 188 bp of E1 gene, as described previously (23, 24). The quality of each DNA sample was verified by the amplification of the 536 bp fragment of ubiquitous human beta-globin gene, as described previously (25, 24). In all HPV PCR positive samples, HPV genotype(s) were determined by restriction fragment analyses of PGMY09/PGMY11 PCR products using seven restriction endonucleases, as described previously (27) or, when necessary, by sequencing either PGMY09/PGMY11 or CPI/IIg PCR products and using the INNO-LiPA HPV Genotyping Assay (Innogenetics, Gent, Belgium).

According to previous hc2/in-house PCRs/HPV genotyping results, 550 samples included in the retrospective evaluation were sorted into four groups: (i) 218 samples were hc2 and PCR positive and contained at least one of 13 high-risk HPV genotypes included in the hc2 high-risk probe cocktail (considered as hc2 true-positive samples), (ii) 259 samples were HPV negative using hc2 as well as both in-house PCRs (considered as hc2 true-negative samples); (iii) 27 samples were hc2 negative but PCR positive and contained at least one of 13 high-risk HPV genotypes included in the hc2 high-risk probe cocktail (considered as hc2 false-negative samples); and (iv) 46 samples were hc2 positive but either negative using both in-house PCRs or PCR positive and contained none of 13 high-risk HPV genotypes included in the hc2 high-risk probe cocktail (considered as hc2 false-positive samples).

All 550 samples included in the retrospective evaluation were tested using the Amplicor HPV test strictly following manufacturer's instructions (21). Briefly, after sample preparation, the 165 bp long part of HPV L1 gene and fragments of the beta-globin gene were co-amplified with a mixture of biotin-labeled primers. Aliquots of denaturated amplicons were added to separate wells of microwell plates coated with either HPV high-risk probes or beta-globin specific oligonucleotide probes. After a washing procedure, bound hybrids were detected with a biotin avidin-horseradish peroxidase assay (21).

Prospective evaluation

In the second part of our study, 312 cervical scrape specimens were tested prospectively for the presence of 13 high-risk HPV genotypes in parallel with hc2 and the Amplicor HPV test. Only the samples with discordant HPV results were additionally tested using PGMY09/PGMY11 and CPI/IIg in-house consensus PCRs, as described above. HPV genotyping was also performed as described above.

Results

Retrospective evaluation

The results of the retrospective part of our evaluation are summarized in Table 1. As shown in Table 1, all 218 hc2-positive samples that contained at least one of 13 high-risk HPV genotypes tested Amplicor positive. Of 259 samples that were negative using hc2 and both in-house PCRs, 257 tested Amplicor negative and two Amplicor positive. One of the hc2-negative/Amplicor-positive samples contained HPV54 and the other HPV54+HPV70; both samples were therefore considered Amplicor false-positive. All 27 hc2-negative samples that contained at least one of the 13 high-risk HPV genotypes and were considered hc2 false-negative samples tested Amplicor positive. Of 46 hc2-positive samples that contained none of the 13 high-risk HPV genotypes and were considered hc2 false-positive samples, 44 samples tested Amplicor negative and two Amplicor positive. Both Amplicor-positive samples contained HPV53 and were therefore considered Amplicor false-positive.

Prospective evaluation

The results of the prospective part of our evaluation are summarized in Table 2. As shown in Table 2, concordant results between hc2 and Amplicor were obtained in 268 (85.9%) of the 312 samples. According to our internal reference HPV standard, all 15 hc2-positive/Amplicor-negative samples were considered hc2 false-positive and Amplicor true-negative. Namely, in none of those 15 samples a high-risk HPV genotype was detected (HPV53 was detected in 4 samples, HPV66, HPV72 and candHPV89 were detected in 2 samples each, and HPV6, HPV10, HPV42, HPV72+HPV81, HPV42+HPV6 in one sample each). According to our internal reference HPV standard, all 29 hc2 negative/Amplicor positive samples contained at least one of the 13 high-risk HPV genotypes and were considered Amplicor true-positive and hc2 false-negative. Thus, HPV45 was detected in 5 samples, HPV51 and HPV52 in 4 samples each, HPV16, HPV 18 in 3 samples each, HPV31 and HPV35 in 2 samples each, HPV31+HPV54, HPV52+HPV6, HPV18+HPV54, HPV16+HPV66, HPV16+HPV52+HPVX and HPV62+HPV52+HPV84 in one sample each.

Discussion

In our study, the analytical performance of a recently developed PCR-based Amplicor HPV test was compared to the FDA-approved hc2 test in a retrospective and prospective manner. Two in-house consensus PCRs, PGMY09/PGMY11 and CPI/IIg, targeting two different HPV genes (L1 and E1, respectively) coupled

Table 2. Results of the prospective evaluation of the Amplicor HPV Test and Hybrid Capture 2 HPV DNA test (hc2) for the detection of high-risk HPV on 312 cervical scrape specimens.

AMPLICOR HPV	**hc2**		
	HPV negative	*HPV positive*	*Total*
HPV negative	176	15*	191
HPV positive	29**	92	121
Total	205	107	312

**According to the internal reference HPV standard, all 15 samples considered as Amplicor true-negative and hc2 false-positive;*
*** According to the internal reference HPV standard, all 29 samples were considered as Amplicor true-positive and hc2 false-negative.*

with HPV genotyping were used as an HPV internal reference standard.

In the retrospective part of our study, 550 samples with previously known hc2 results and resolved HPV status were tested using Amplicor. As expected, the Amplicor results agreed almost completely with the HPV internal reference standard results (Table 1). Since in the samples with discordant hc2 and internal reference standard results (false-reactive hc2 samples), Amplicor mostly tested in concordance with internal reference standard results, a higher analytical sensitivity and specificity of Amplicor in the detection of 13 high-risk HPV genotypes in comparison to hc2 is anticipated. However, since the retrospective evaluation was performed on pre-selected samples with an artificially high rate of samples with hc2 false-reactive results and since our internal reference standard was PCR-biased, the exact analytical sensitivity and specificity of Amplicor cannot be calculated.

To establish an exact concordance between the HPV results generated by Amplicor and hc2, a prospective evaluation of both tests followed. In the prospective evaluation performed on 312 samples in the settings of a routine diagnostic laboratory, concordant Amplicor and hc2 results were obtained in 85.9% of tested samples (Table 2). In comparison, in a recent study by Perrons et al., which compared another commercial PCR-based assay SPF_{10}-LiPA System (Innogenetics, Gent, Belgium) with hc2, the concordance between the results of two tests was 70.3% (28). According to our internal reference HPV standard, all 44 samples with discordant hc2 and Amplicor results were considered as hc2 false-reactive and Amplicor true-reactive samples. Similar results were also obtained after additional analysis of samples with discordant results in the study of Perrons et al. (28).

As previously mentioned, the exact analytical sensitivity and specificity of Amplicor cannot be reliably calculated from our study, but it is obvious from our results that both analytical parameters are higher for Amplicor than they are in hc2. Although higher *analytical* sensitivity and the specificity of a particular diagnostic test do not automatically translate into higher *clinical* sensitivity and specificity, we are convinced that the demonstrably higher analytical specificity of Amplicor in comparison to that of hc2 can be considered clinically beneficial since Amplicor certainly produces less false positive results than hc2, which would consequently reduce the costs and unnecessary management of women with HPV high-risk false-positive results. In contrast, the clinical importance of the higher analytical sensitivity of PCR-based HPV detection methods, like Amplicor, in comparison to hc2 is still a matter for extensive professional discussion (29). As has been reviewed recently, some authors hypothesized that high HPV viral load seems to be indicative of viral persistence and disease development, while low HPV viral load is associated with the clearance of an infection and even regression of cervical lesions (29). Since PCR-based methods are analytically more sensitive than hc2, hc2-negative/Amplicor-positive samples are most probably the samples with low HPV viral load. However, when one evaluates the low HPV viral load detected in a particular cervical scrape specimen, two important facts should be kept in mind: (i) swabing of the cervix which is the usual way of obtaining cervical specimens is NOT a quantitative collection of a clinical sample and low amount of HPV detected in a particular specimen can be also the result of a poor quality specimen containing a low amount of cervical epithelial cells and (ii) the presence of HPV infection (irrespective of viral quantity) indicates only a risk of having or developing a HPV-related cervical lesion and is not equivalent to a morphological disorder. Therefore, sensitive methods to diagnose HPV infection may be required in order to realize a maximum negative predictive value for the development of HPV-associated cervical lesions, particularly when HPV is used for triaging patients with ASC-US (21).

In conclusion, in our retrospective and prospective study, the Amplicor HPV test demonstrated a high analytical sensitivity and specificity. The higher analytical specificity of Amplicor in comparison to that of hc2 can be considered clinically useful. However, prospective studies with clinical endpoints like CIN2+ are urgently needed to assess the clinical utility of the higher analytical sensitivity of PCR-based methods like the Amplicor HPV test for primary HPV screening and triaging patients with ASC-US.

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