The Abbott RealTime High Risk HPV test: comparative evaluation of analytical specificity and clinical sensitivity for cervical carcinoma and CIN 3 lesions with the Hybrid Capture 2 HPV DNA test

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ABSTRACT

Background: The Abbott RealTime High Risk HPV test (RealTime) is a novel assay designed to detect 14 high-risk human papillomavirus genotypes (hr-HPV) and concurrently distinguish HPV-16 and HPV-18 from other hr-HPV within a single test.

Objective: To evaluate analytical specificity and clinical sensitivity for cervical carcinoma and cervical intraepithelial neoplasia grade 3 (CIN3) of the RealTime test in comparison with the Digene Hybrid Capture II Test (hc2).

Materials and methods: Analytical specificity of the RealTime assay was evaluated on 37 samples with previously determined hc2 false-positive results due to cross-reactivity of the hc2 high-risk probe cocktail with untargeted low-risk HPV genotypes. All 37 samples were negative for 14 hr-HPV using the RealTime test. Clinical sensitivity of RealTime was evaluated in comparison to hc2 on 95 and 267 archived routine cervical specimens collected from women with histologically confirmed cervical carcinoma and CIN3 lesions, respectively. Archived specimens were selected for the present study after linkage with the Slovenian national registry of CIN3 and cervical cancer to obtain histology data.

Results: Concordant results between RealTime and hc2 were obtained in 90/95 cervical cancer samples (94.7% agreement) and in 250/267 CIN3 samples (93.6% agreement). Clinical sensitivity of RealTime and hc2 for cervical cancer in the total study cohort was 88.4% (95% confidence interval (CI): 80.3–93.6%) and 87.3% (95% CI: 79.0–92.8%), respectively, and analytical sensitivity for samples containing at least one targeted hr-HPV was 98.8% (95% CI: 93.0–100.0%) and 95.3% (95% CI: 88.2–98.5%), respectively. Clinical sensitivity of RealTime and hc2 for CIN3 lesions of the total study cohort was 91.8% (95% CI: 87.8–94.5%) and 89.1% (95% CI: 84.8–92.3%), respectively, and analytical sensitivity for samples containing at least one targeted hr-HPV was 96.4% (95% CI: 93.3–98.2%) and 92.5% (95% CI: 88.5–95.2%), respectively.

Conclusion: The RealTime test showed excellent analytical specificity and no cross-reactivity with low risk HPV genotypes that tested positive with hc2. Clinical sensitivity of the RealTime assay using archived routine cervical specimens was comparable to hc2. The RealTime test is an important new method applicable to cervical carcinoma screening and management of cervical precancerous lesions.

KEY WORDS

HPV, Abbott, Hybrid Capture, real-time PCR, CIN 3, cervical cancer
Introduction

Human papillomaviruses (HPVs) are remarkably diverse DNA viruses that are causally involved in the etiology of various benign and malignant neoplastic lesions of mucosal and skin epithelium (1). More than 200 HPV genotypes have currently been described, of which 115 have been officially designated and taxonomically defined (2, 3). Forty different HPV genotypes from the alpha genus are known to infect the mucosal epithelium, with a subset of at least 15 genotypes being associated with lesions that can progress to cancer (2, 4). These cancer-associated HPV genotypes are designated as high-risk HPV (hr-HPV) genotypes and are the etiological agents of virtually all cervical carcinomas (1, 5). The clinically most important hr-HPV genotypes are HPV-16 and HPV-18, found in 50 to 65% and 7 to 20% of cases of cervical cancer, respectively (6). In addition to cervical carcinoma, hr-HPV genotypes, the most frequent being HPV-16, play the leading etiological role in the development of anal cancer and substantial proportion of vaginal, penile, vulvar, and oropharyngeal (mainly tonsillar) cancers (7–10).

Detecting hr-HPV has recently become an important part of cervical carcinoma screening and detection algorithms (11, 12). Five years ago the US Food and Drug Administration (FDA) approved concurrent HPV and Pap smear screening of women 30 years and older. In addition to primary screening, several consensus guidelines recommend HPV testing for (i) triage in cases of equivocal cytology results showing the presence of atypical squamous cells of undetermined significance (ASC-US) to determine which patients should be referred for colposcopy and (ii) for follow-up after treatment of precancerous cervical lesions (11–14). For these screening purposes, a range of in-house and commercial molecular tests have been developed in order to distinguish hr-HPV infections from no HPV infection (11). The Hybrid Capture II Test (hc2) originally developed by the Digene Corporation (Gaithersburg, MD) and currently marketed by Qiagen (Hilden, Germany), is the most frequently used HPV screening test at present. hc2 is a solution-based hybridization assay designed to detect 13 hr-HPV genotypes (HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, HPV-51, HPV-52, HPV-56, HPV-58, HPV-59, and HPV-68) using a mixture of unlabeled full genomic length RNA probes (15). Although several studies have clearly demonstrated that hc2 is a sensitive, specific, and reliable test for detecting clinically relevant HPV infections, some recently conducted studies showed a significant analytical inaccuracy of hc2, mainly due to the cross-reactivity of its probe cocktail outside the spectrum of 13 targeted hr-HPV genotypes (16–18).

Abbott RealTime High Risk HPV test (Abbott Molecular Inc., Des Plaines, IL) based on real-time polymerase chain reaction (PCR) was recently introduced in Europe. The Abbott RealTime High Risk HPV test (RealTime) is performed on the m2000rt real-time PCR instrument (Abbott Molecular) and is designed to identify 14 hr-HPV genotypes (HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, HPV-51, HPV-52, HPV-56, HPV-58, HPV-59, HPV-66 and HPV-68) using a modified GP5+/6+ primer mix consisting of three forward and two reverse primers (19). The assay uses four channels for detecting fluorescent probes; one for detecting the internal control (human beta-globin), a second one for detecting HPV-16, a third one for detecting HPV-18, and a fourth one for detecting the remaining 12 hr-HPV genotypes. The assay turnaround time is 6 to 8 hours for 96 samples and depends on the method used for DNA extraction (19). The fully automated high throughput m2000sp instrument (Abbott Molecular Inc.) or smaller m24sp instrument (Abbott Molecular Inc.) can be used for DNA extraction, or alternatively DNA can be prepared manually. The RealTime assay is currently validated for use with cervical specimens collected in PreservCyt solution (Hologic, Marlborough, MA) only (19).

To the best of our knowledge, three studies evaluating various aspects of RealTime have been published in peer-reviewed journals to date by manufacturer’s researchers (19–21). The first study described design principles and evaluated analytical performance of RealTime (19). Probit analysis showed that the analytical sensitivity of RealTime was between 500 and 5,000 copies of HPV DNA per assay, depending on the genotype (19). An analytical specificity study on a panel of 41 bacteria, viruses, and fungi, including 15 low-risk HPV genotypes and other microorganisms that can be found in the female anogenital tract, revealed no cross-reactivity with any of the organisms tested (19). Interference or PCR-inhibition was not observed with any of the tested substances that may be present in cervical specimens (19). In the second study, clinical sensitivity of the RealTime test was evaluated on archived cervical specimens from Amsterdam and the presence of hr-HPV was detected in 97.2% (246/253) and 98.5% (335/340) of cervical intraepithelial neoplasia grade 3 (CIN3) and cancer specimens, respectively (20). In specimens from women 30 years or older with...
normal cytology in a screening population, the HPV positivity rate was 6.5% (49/757) (20). In the third study, RealTime demonstrated similar clinical performance detecting CIN2+ and CIN3+ when compared to hc2 on 702 patients with abnormal cytology referred to colposcopy (21). The clinical sensitivity for detecting CIN2+ was shown to be 97.8% with RealTime and 95.6% with hc2, and for detecting CIN3+ 100% with RealTime and 97% with hc2 (21).

This study evaluated the analytical specificity and clinical sensitivity of RealTime for cervical carcinoma and CIN3 lesions in comparison with the FDA-approved hc2 test in the settings of a routine virological laboratory. Due to the fact that the analytical performance of RealTime has been evaluated in detail in a previous study (19), our analytical evaluation of RealTime was performed only on a subset of 37 samples with previously determined false-positive hc2 results. The clinical sensitivity of RealTime was evaluated in comparison to hc2 on a total of 362 archived routine cervical specimens collected from women with histologically confirmed cervical carcinoma or CIN3 lesions.

Materials and Methods

Analytical specificity

Thirty-seven archived routine cervical specimens collected in 1 ml of Digene Specimen Transport Medium (STM; Qiagen, Gaithersburg, MD) with previously determined hc2 hr-HPV false-positive results were included in the study. All 37 samples tested hc2 hr-HPV–positive using a high-risk probe cocktail with relative light unit per cutoff (RLU/CO) values between 1.1 and 4.0, but were negative for 14 hr-HPV genotypes (13 hr-HPV genotypes targeted in the hc2 plus HPV-66) using three commercial assays: the Amplicor HPV Test (Roche Diagnostics, Branchburg, NJ), the INNO-LiPA HPV Genotyping Extra Test (Innogenetics, Gent, Belgium), and the Linear Array HPV Genotyping Test (Roche Diagnostics). Because the presence of only low-risk HPV genotypes was detected in all 37 specimens after extensive testing using the INNO-LiPA HPV Genotyping Extra Test, the Linear Array HPV Genotyping Test, HPV-6/-11/-42/-43/-44/-55 in-house real-time PCR assays (22, 23), and sequencing of GP5+/GP6+ and PGMY09/11 PCR-products (24), all 37 samples were considered hc2 hr-HPV false-positive due to the cross-reactivity of the hc2 high-risk probe cocktail with untargeted low-risk HPV genotypes (16, 17). For this study, 200 μl aliquots of the specimens collected in STM were removed immediately upon arrival of specimens in the laboratory and prior to the addition of the hc2 denaturing reagent solution (the first step of hc2 testing). Specimens were stored at −70 °C. Prior to sample extraction, 20 μl of the specimens was spiked to mLysis Buffer (Abbott Molecular Inc.) and processed on the m2000sp and m2000rt instruments, following the manufacturer’s instructions (19).

Clinical sensitivity for cervical carcinoma and CIN3 lesions

The clinical sensitivity of RealTime for cervical carcinoma and CIN3 lesions was evaluated in comparison to hc2 on a total of 362 archived routine cervical specimens. No samples were collected solely for the purpose of this study. The samples included in the study were selected from a total of 8,967 samples, which were routinely tested in our laboratory between 2005 and 2008 for the presence of hr-HPV using hc2. Sample inclusion was based on the ability to link with the Slovenian national registry of CIN3 and cervical cancer to obtain histology data. Only samples taken within 1 year before entering the national registry of CIN3 and cervical cancer were included in the study. In total, 95 and 267 samples collected in women with histologically confirmed cervical carcinoma and CIN3 lesion, respectively, were included in the study. Samples had originally been collected in 1 ml of STM by approximately 70 different public or private practices for various reasons (clinician-based decision). For this study, 500 μl aliquots of STM were removed immediately upon arrival of the specimens in the laboratory, out of which 20 μl was used for RealTime test as described above, and the remaining aliquots were tested by hc2 after addition of hc2 denaturing reagent solution, following the manufacturer’s instructions (16). The two aliquots of one clinical sample kept at −70 °C were tested in parallel within 1 week using the two comparator assays.

The INNO-LiPA HPV Genotyping Extra Test was used as the main test for resolving discordance between RealTime and hc2, as described previously (25). The INNO-LIPA test is capable of recognizing 27 different alpha-HPV genotypes. It specifically identifies all hr-HPV genotypes covered by RealTime and hc2, two additional hr-HPV genotypes (HPV-73 and HPV-82), and two probable hr-HPV genotypes (HPV-26 and HPV-53).

All samples negative for both tests and some samples with discordant results were further tested.
**Table 1. Results of comparative evaluation of RealTime and hc2 on 95 archived routine cervical specimens collected from the same number of women with histologically confirmed cervical carcinoma.**

<table>
<thead>
<tr>
<th></th>
<th>hc2 hr-HPV–positive</th>
<th>hc2 hr-HPV–negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>RealTime hr-HPV–positive</td>
<td>81</td>
<td>3*</td>
<td>84</td>
</tr>
<tr>
<td>RealTime hr-HPV–negative</td>
<td>2**</td>
<td>9***</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>83</td>
<td>12</td>
<td>95</td>
</tr>
</tbody>
</table>

*All three samples tested hr-HPV–positive by the INNO-LiPA HPV Genotyping Extra Test method.*

**Results**

**Analytical specificity**

All 37 samples with previously determined hc2 hr-HPV false-positive results due to cross-reactivity of the hc2 high-risk probe cocktail with untargeted low-risk HPV genotypes tested as hr-HPV–negative using RealTime. The results obtained with RealTime were in full concordance with previously obtained hr-HPV–negative results using the Amplicor HPV Test, INNO-LiPA HPV Genotyping Extra Test, and Linear Array HPV Genotyping Test in the same collection of samples.

**Clinical sensitivity for cervical carcinoma**

The results of comparative evaluation of clinical sensitivity of RealTime and hc2 for histologically confirmed cervical carcinoma performed on 95 archived routine cervical specimens are summarized in Table 1. As shown in Table 1, concordant results were obtained from 90 out of 95 samples (94.7% agreement). Nine samples with concordant RealTime and hc2 HPV-negative results were additionally tested using three commercial HPV assays and three in-house PCRs, and 8 out of 9 samples tested HPV-negative using all six additionally applied PCR-based HPV methods. The presence of HPV-16 in very low quantity was confirmed in one sample.
Table 2. Results of comparative evaluation of RealTime and hc2 on 267 archived routine cervical specimens collected from women with histologically confirmed CIN3 lesions.

<table>
<thead>
<tr>
<th></th>
<th>hc2 hr-HPV–positive</th>
<th>hc2 hr-HPV–negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>RealTime hr-HPV–positive</td>
<td>233</td>
<td>12*</td>
<td>245</td>
</tr>
<tr>
<td>RealTime hr-HPV–negative</td>
<td>5**</td>
<td>17***</td>
<td>22</td>
</tr>
<tr>
<td>Total</td>
<td>238</td>
<td>29</td>
<td>267</td>
</tr>
</tbody>
</table>

*All 12 samples tested positive by the INNO-LiPA HPV Genotyping Extra Test method.
**2 samples hr-HPV–positive and 3 samples hr-HPV–negative in discordant testing.
***10 out of 17 samples tested HPV-negative using six additional PCR-based HPV methods and the presence of at least one targeted hr-HPV genotype was detected in 7 samples.

Clinical sensitivity for CIN3 lesions

The results of comparative evaluation of the clinical sensitivity of RealTime and hc2 for histologically confirmed CIN3 lesions performed on 267 archived routine cervical specimens are summarized in Table 2. As shown in Table 2, concordant results were obtained in 250 out of 267 samples (93.6% agreement). Similar to the cervical cancer group, 17 samples with concordant RealTime and hc2 HPV-negative results were additionally tested using three commercial HPV assays and three in-house PCR methods. Ten out of 17 samples tested HPV-negative using all six additional PCR-based HPV methods. The presence of at least one targeted hr-HPV genotype was determined in the remaining 7 samples: HPV-51 and HPV-33 in two samples each and HPV-16, HPV-35, and HPV-39+HPV-6 in one sample each. Interestingly, 4 out of 7 samples containing at least one targeted hr-HPV genotype, which tested negative using RealTime, showed HPV-specific amplification in late PCR cycles but were determined as RealTime HPV “not detected” due to the manufacturer’s cut-off criteria. As shown in Table 2, 12 samples tested RealTime-positive and hc2-negative. In all 12 RealTime-positive/hc2-negative samples, the presence of hr-HPV genotypes was confirmed using the INNO-LiPA HPV Genotyping Extra Test: seven samples contained HPV-16, two samples contained HPV-51, and one sample each contained HPV-18, HPV-52, and HPV-31+HPV-33. The RealTime assay accurately grouped HPV genotypes into HPV-16, HPV-18, and other hr-HPV groups in all 12 samples. According to our interpretation criteria, all 12 RealTime-positive/hc2-negative samples were considered analytically and clinically RealTime hr-HPV false-negative. As shown in Table 2, five samples tested RealTime-negative and hc2-positive. Further testing of discordant samples revealed the presence of targeted hr-HPV in two samples containing HPV-31 and HPV-58 each and the presence of untargeted low-risk HPV-67 in two samples, and in one sample HPV was not detected by any of the six additional PCR-based HPV methods. According to our interpretation criteria, two out of five RealTime-negative/hc2-positive samples were considered analytically and clinically RealTime false-negative, two samples were interpreted to be hc2 analytically false-positive but clinically true-positive, and one sample was considered analytically RealTime true-negative – but according to the clinical diagnosis as clinically RealTime false-negative. Taking all of the results together, the clinical sensitivity of RealTime and hc2 for CIN3 lesions in the total study cohort was 91.8% (95% CI: 87.8–94.5%) and 89.1% (95% CI: 84.8–92.3%), respectively, and analytical sensitivity of RealTime and hc2 for samples containing at least one targeted hr-HPV (after exclusion of 13 samples in which the presence of hr-HPV DNA could not be detected with any of six additionally applied PCR-based HPV methods) was 96.4% (95% CI: 93.3–98.2%) and 92.5% (95% CI: 88.5–95.2%), respectively.

Discussion

Detection of hr-HPV has recently become an important part of cervical carcinoma screening and detection algorithms (11, 12). Most hr-HPV infections are spontaneously cleared by the host immune system and only about 3 to 10% of hr-HPV–infected women eventually develop cervical cancer or other carcinomas in the anogenital region (4). As a result, a clinically useful HPV test needs to be designed with optimal clinical sensitivity and specificity in order to effectively
detect high-grade cervical disease and at the same time minimize the number of positives associated with highly prevalent transient HPV infections (19, 28). The recently developed RealTime test is the first commercially available test that utilizes real-time amplification technology to detect 14 hr-HPV types and concurrently type HPV-16 and HPV-18. It was designed to meet emerging needs in cervical carcinoma screening and management of cervical precancerous lesions. Namely, although for almost two decades we have mainly relied on HPV DNA-based screening tests capable of detecting hr-HPV infection (hc2 being a prototype of such tests), recent studies have clearly shown that HPV-16 and HPV-18 are remarkably powerful human carcinogens that are different from other hr-HPV genotypes in a way that merits separate laboratory and clinical consideration. HPV-16 and HPV-18 showed significantly lower clearance rates in comparison with other hr-HPV (29) and remarkable enrichment in high-grade cervical lesions and cancer (30). Thus, infection with HPV-16 and HPV-18 together can be identified in 2 to 4% of women with normal cytology, 6 to 27% of ASC-US lesions, 16 to 32% of low-grade squamous intraepithelial lesions (LSIL), 41 to 67% of high-grade squamous intraepithelial lesions (HSIL), and 65 to 75% of cervical carcinoma (30). A recent study of 1,213 cases of carcinoma in situ and 808 invasive cervical cancers diagnosed from 1980 to 1999 in New Mexico (USA) showed that the age at diagnosis of HPV-16 and HPV-18–related cancers was 5 years earlier than that of cancers caused by other hr-HPV genotypes (48.1 years [46.6–49.6 years] vs. 52.3 years [50.0–54.6 years]) (31). However, the most striking evidence for the remarkably powerful carcinogenic potential of HPV-16 and HPV-18 comes from three prospective studies measuring cumulative incidence of CIN2+ lesions, which showed the significantly higher progression risks associated with HPV-16 and HPV-18 in comparison to other hr-HPV in women with normal cytology in a general screening population (32–34). Based on these data, the American Society for Colposcopy and Cervical Pathology updated their 2006 Consensus Guidelines in March 2009, recommending that for cytology-negative women 30 years and older that are hr-HPV DNA-positive (for any of the hr-HPV genotypes) molecular genotyping assays that detect HPV-16 and HPV-18 would be clinically useful for determining which women should be referred for immediate colposcopy (HPV-16 or HPV-18–positive), and which could be followed-up with repeat cytology and hr-HPV testing in 12 months (hr-HPV–positive but HPV-16 or HPV-18–negative).

Although hc2 is the most widely used HPV test and many studies have demonstrated that hc2 is a sensitive and reliable test for detecting clinically important HPV infections, some recent studies have shown a significant analytical inaccuracy of hc2, mainly due to the cross-reactivity of its probe cocktails. Thus, in 2002, our research group determined the specificity of the hc2 high-risk probe cocktail by genotyping of HPV isolates obtained from 325 women recognized as HPV-positive using the high-risk probe cocktail (35). Genotyping of Slovenian samples showed that, in addition to the genotypes included in the high-risk probe cocktail, the hc2 high-risk probe cocktail detects several other HPV genotypes. These results were also confirmed by other research groups and, currently, it has been established that, in addition to 13 HPV genotypes included in the high-risk probe cocktail, the hc2 high-risk probe cocktail detects at least 28 other HPV genotypes, many of them classified as low-risk HPV genotypes. A recent genotyping study on 3,179 specimens from women participating in the ALTS trial (a clinical trial to evaluate management strategies for women with ASC-US or LSIL) found that 7.8% of all hc2-positive results in this population were false-positive due to the cross-reactivity of hc2 with untargeted, noncarcinogenic HPV genotypes (18). Our research group recently showed that the probability of hc2 false-positive results increased with the proximity to the hc2 cut-off value (17). According to these results, we recommended the introduction of an hc2 gray zone and retesting of samples with repeatedly borderline hc2 results (samples with RLU/CO values between 1.0 and 4.0) by an alternate detection method (17). At present, in Slovenia we routinely test all cervical samples for HPV using the hc2 high-risk cocktail. The samples with hc2 RLU/CO values less than 1.0 are considered hr-HPV DNA-negative and samples with hc2 RLU/CO values greater than 4.0 are considered hr-HPV DNA-positive, without any additional testing. In contrast to the manufacturer’s recommendations, but based on our findings, all samples in the hc2 RLU/CO ranging between 1.1 and 4.0 (approximately 5% of all samples) are additionally tested using the Amplicor HPV Test (27), and final hr-HPV results are released according to the results of the Amplicor HPV Test (17). Our unique routine testing strategy and the fact that analytical inaccuracy of hc2 is currently accepted as the main disadvantage of the hc2 test were the main reasons why we analytically evaluated RealTime only on the subset of 37 samples with previously determined hc2 false-positive results. As expected, all 37 samples with previously determined hc2 hr-
HPV false-positive results due to cross-reactivity of the hc2 high-risk probe cocktail with untargeted low-risk HPV genotypes tested negative for 14 hr-HPV using RealTime, confirming the excellent analytical specificity of this test established in a previous study (19).

High clinical sensitivity for cervical carcinoma and CIN3 lesions is one of the main comparative advantages of HPV tests over cytology. Two studies have been published in peer-reviewed journals to date evaluating the clinical sensitivity of RealTime for cervical carcinoma or CIN3 lesions (20, 21), both of which showed higher clinical sensitivity than what was observed here. In one of the studies, the clinical sensitivity of RealTime was evaluated on archived cervical specimens from Amsterdam and the presence of hr-HPV was detected in 97.2% (246/253) and 98.9% (335/340) of histologically confirmed CIN3 and cancer specimens, respectively (20). In the other study, clinical sensitivity for CIN3+ specimens from a colposcopy referral population due to abnormal cytology was 100% (21). Although virtually all cervical carcinomas and CIN3 lesions should be hr-HPV–positive (5, 35), such a high detection rate is rarely achieved in the “real world” situation due to several (mainly technical) problems, including specimen quality. Thus the most probable reason for lower sensitivity in our study is suboptimal quality of some of our specimens. The specimens included in our study were not collected in strictly controlled clinical conditions (as is usually done in clinical trials), but were archived routine cervical specimens selected for this study after linkage with the Slovenian national registry of CIN3 and cervical cancer to obtain histology data. Samples were collected by approximately 70 different public or private practices for various reasons. Similar to the majority of other countries, no organized quality control program of sample collection exists in Slovenia. Because 18 out of 26 (69.2%) specimens that were hr-HPV–negative using both RealTime and hc2 were also HPV-negative using six additionally applied PCR-based HPV methods, we believe that the suboptimal quality of these specimens was the most probable reason for clinically false-negative results. Another possible reason for the lack of detection for CIN3 and cancer specimens is that some of the specimens had very low levels of HPV DNA despite having high-grade disease. Among 26 specimens negative by both tests, 6 specimens had HPV specific amplification in the RealTime test with a cycle number beyond the assay cut-off cycle, indicating the presence of very low levels of HPV target that may not be reliably detected. In addition histological misclassification is also a possible reason for the discrepancy of histology and hr-HPV results in 26 samples. Although histology is performed exclusively by certified specialists in clinical pathology in Slovenia and there is an organized national quality control program for cervical pathology and cytology, a certain degree of inter-observer variability is always present even in the best pathology departments (36, 37). An additional histological review of tissue slides in patients that tested HPV DNA-negative could resolve the dilemma, but due to strict standards of patients’ medical records protection in our country we were not allowed to perform such an additional review.

Several previously published studies comparatively evaluated clinical and analytical sensitivity of the FDA-approved hc2 test with other non-FDA-approved PCR-based HPV assays introduced to the diagnostic market in the past few years (28). As shown in Tables 1 and 2, concordance between hc2 and RealTime was similar to levels obtained between hc2 and the majority of other non-FDA-approved PCR-based assays evaluated to date (28). Testing of samples with discordant results showed that all 15 RealTime-positive/hc2-negative samples (Tables 1 and 2) represent hc2 false-negative results. By additional testing of seven samples which tested RealTime negative and hc2 positive, targeted hr-HPV was detected in two samples, untargeted low-risk HPV-67 was found in two samples, and no HPV of any genotype could be identified in three samples. Our study confirms that, paradoxically, some level of analytical inaccuracy of the HPV test (in this case of hc2) can be clinically beneficial. Namely, although detecting low-risk HPV-67 in two samples using the hc2 high-risk cocktail should be considered an analytically inaccurate result, cross-reactivity of the hc2 high-risk probe cocktail with untargeted low-risk HPV genotypes (described in detail above) helped hc2 identify two women with CIN3 lesion.

In conclusion, in our hands the RealTime assay showed excellent analytical specificity and the absence of cross-reactivity with untargeted HPV genotypes that had resulted in hc2 false-positive results. The clinical sensitivity of RealTime evaluated on 362 archived routine cervical specimens collected from women with histologically confirmed cervical carcinoma or CIN3 lesions was comparable to hc2. The RealTime assay, designed to detect 14 hr-HPV genotypes and concurrently type HPV-16 and HPV-18 within a single test, is an important new diagnostic tool in cervical carcinoma screening and management of cervical precancerous lesions.
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