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Prospective Evaluation of the Hybrid Capture 2 and AMPLICOR Human Papillomavirus (HPV) Tests for Detection of 13 High-Risk HPV Genotypes in Atypical Squamous Cells of Uncertain Significance[▽]

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The use of high-risk human papillomavirus (hrHPV) testing as an adjunct to cervical cytology in population-based screening programs is currently based on DNA hybridization and PCR assays. The aim of this study was to prospectively assess the diagnostic performance of the Hybrid Capture 2 test (HC2; Digene Corporation) in comparison with that of the recently developed PCR-based AMPLICOR HPV test (Roche Molecular Systems) for the detection of 13 hrHPV types. A reverse line blot hybridization assay (Innogenetics) was used as an internal reference standard in discordant cases. Two hundred seventy-one patients with atypical squamous cells of uncertain significance (ASCUS) in cervical samples underwent hrHPV testing. The chi-square test was performed to compare respective proportions. Totals of 160/271 (59%) and 156/271 (58%) were found to be positive for hrHPV with HC2 and AMPLICOR, respectively. Concordant results were obtained for 235 (86.7%) of the 271 samples (kappa statistic, 0.73 ± 0.04). Considering types 26, 53, and 66 as oncogenic types, negative predictive values (NPVs) of HC2 and AMPLICOR were 92.8% and 87.8%, respectively (difference was not significant), and their respective accuracies were 94.8% and 91.9% (difference was not significant). Considering types 26, 53, and 66 as not oncogenic, the respective HC2 and AMPLICOR NPVs were 92.8% and 97.4% (difference was not significant), and accuracy was significantly higher for the AMPLICOR assay (95.9% versus 90.8% for HC2) ($P < 0.05$). For ASCUS samples, the NPV was 92.8% for HC2 testing and might be compromised if the copy number of HPV DNA was low. The NPV was 97.4% for the AMPLICOR assay and might be compromised if HPV types 26, 53, and 66 were considered oncogenic. The accuracy of these two assays is good and is compatible with routine clinical use in the triage of ASCUS cases.

The U.S. Food and Drug Administration (FDA) recently approved concurrent human papillomavirus (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 (ASCUS) (18, 22). The Hybrid Capture 2 HPV DNA test (HC2; Digene Corporation, Gaithersburg, MD), a microplate-based solid-phase hybridization assay for the detection of 13 high-risk HPVs (hrHPVs) (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68), 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 (1, 8, 21). Although HC2 is the most widely used HPV test and is a sensitive and reliable test for the detection of HPV (4, 9–11, 16, 17), several recent studies showed a significant analytical inaccuracy of the HC2 test near the cutoff, mainly due to the cross-reactivity of its high-risk probe cocktail (4, 9–11, 16, 17).

Snijders et al. defined "analytical" and "clinical" sensitivity and specificity in order to distinguish clinically irrelevant and clinically relevant HPV positivity rates (19). Analytical sensitivity is defined as the proportion of HPV-positive women who are correctly identified by a given test (but clinically unimportant), whereas clinical sensitivity identifies the proportion of women with disease (i.e., women with cervical intraepithelial neoplasia grade 3 or higher) who are correctly identified by a positive HPV test. However, note that HPV infection indicates a risk of having or developing a cervical lesion and is not equivalent to a morphological disorder. Although the natural history of HPV infections is not fully understood, the viral load is probably low during the first phase of infection and increases over time, in parallel with the development of cytological disorders (19). Therefore, sensitive methods to diagnose HPV infection are needed to have a high negative predictive value for HPV-associated cervical carcinoma, particularly since most cervical smears are classified as normal or ASCUS. Indeed, for HPV testing to be introduced into cervical cancer prevention programs, HPV tests must be standardized, reliable, and accurate.

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In mid-2004, the PCR-based AMPLICOR HPV test (Roche Molecular Systems, Branchburg, NJ) was launched on the European market. The AMPLICOR 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 for high-risk HPV screening. The newly developed AMPLICOR test has been validated in a clinical laboratory setting in comparison with the INNO-LiPA test (Innogenetics), with good agreement between the methods (20). Poljak et al. reported a concordance rate of 85.9% between HC2 and AMPLICOR assays in a retrospective and prospective study (17).

In 2003, the IARC proposed an epidemiological classification of the HPVs regarding their oncogenic potential: 15 HPV types were classified as high-risk types (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82), 3 were classified as probable high-risk types (types 26, 53, and 66), and 12 were classified as low-risk types (types 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108) (14).

In the present study, we evaluated prospectively the analytical performance of HC2 in comparison with that of AMPLICOR with regard to the ability to reliably detect 13 high-risk HPV genotypes, i.e., HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, and HPV68, in the setting of a routine diagnostic laboratory with ASCUS clinical situations.

MATERIALS AND METHODS

Patients. The inclusion criterion was patients with ASCUS. A screen of approximately 5,000 patients tested for HPV during the year 2004 was assessed in five centers in France (Marseille, Aix les Bains, Lyon, Le Havre, and Meylan). Two hundred seventy-one patients with ASCUS were included and underwent high-risk HPV testing of cervical samples.

Cytology. Cervical samples for liquid-based cytology (LBC) were collected by use of a specially designed sampling device, which was rinsed in the LBC medium PreservCyt (ThinPrep liquid Pap vial; Cytyc Corporation) and prepared for ThinPrep specimens following the manufacturer's recommendations.

High-risk HPV testing. hrHPV detection was done using two assays, namely, HC2 and AMPLICOR. Samples were collected in PreservCyt LBC medium (ThinPrep liquid Pap vial; Cytyc Corporation). Before being processed for the HPV DNA tests, samples were maintained between 2°C and 30°C for up to 3 weeks. Samples were mixed before collection of the volume of sample necessary to do the tests. hrHPV testing was performed on the same swab sample for each patient.

(i) HC2 assay (Digene). HPV testing was performed on a routine basis by using the HC2 assay according to the manufacturer's instructions. A test was considered positive for the presence of hrHPV DNA if the number of relative light units obtained from the luminometer equaled or exceeded the mean of the three positive control values (cutoff of 1.0 pg ml^{-1}). A relative light unit measurement of <1.0 pg ml^{-1} indicated either the absence of the 13 hrHPV types or HPV DNA levels below the threshold of detection. Nonspecific hybridization is characteristic of types 11, 53, 54, 55, 66, MM4, MM7, MM8, and MM9.

(ii) AMPLICOR HPV PCR (Roche). For DNA extraction, an AmpliLute liquid medium extraction kit from Roche was used. For PCR amplification, a pool of HPV primers was designed to amplify HPV DNAs from 13 high-risk genotypes (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). Capture probe sequences are specific for polymorphic regions of L1 bound by these primers. An additional primer pair targets the human β-globin gene (268-bp amplicon) to provide a control for cell adequacy, extraction, and amplification. AmpliTaq Gold DNA polymerase extends the annealed primers along the target templates to produce an approximately 165-bp double-stranded HPV target DNA molecule or a 268-bp β-globin DNA amplicon.

For hybridization after PCR amplification, the HPV amplicon and the β-globin amplicon are chemically denatured to form single-stranded DNA by the addition of denaturation solution. The biotin-labeled HPV and β-globin amplicons are hybridized to the oligonucleotide probes bound to the wells of a multiwell plate (MWP). This hybridization of amplicons to the probes increases the overall specificity of the test.

For detection following the hybridization reaction, the MWP is washed to remove unbound material, and avidin-horseradish peroxidase conjugate is added to each well of the MWP. The conjugate binds to the biotin-labeled amplicon hybridized to oligonucleotide probe 2 (HPV or β-globin) bound to the MWP. According to the manufacturer's specifications, the AMPLICOR test can detect HPV genotypes 31, 52, 58, and 59 at 240 copies/ml and HPV genotypes 16, 18, 33, 35, 39, 45, 51, 56, and 68 at 100 copies/ml, with a positivity rate of >95%. All genotypes are detected with a 100% positivity rate at 480 copies/ml.

Analysis of discordant results. When discordant results were observed between HC2 and AMPLICOR, we used the INNO-LiPA HPV test, a reverse line blot hybridization (Innogenetics) assay, as a reference standard. The INNO-LiPA HPV detection/genotyping assay is capable of detecting and genotyping 25 HPV types simultaneously and has proved to be sensitive, specific, simple, and rapid in the assessment of HPV.

INNO-LiPA HPV detection and genotyping. (i) PCR amplification of HPV DNA. The QIAamp DNA blood method was used to extract DNA. Broad-spectrum HPV DNA amplification was performed using a short PCR fragment assay (INNO-LiPA HPV detection/genotyping assay [SPF_{10} system, version 1]; Labo Biomedical Products bv, Rijswijk, The Netherlands). This assay amplifies a 65-bp fragment of the L1 open reading frame and allows detection of at least 43 HPV types. The SPF_{10} PCR was performed with a final reaction volume of 50 μl containing 10 μl of the isolated DNA sample, 10 mmol/liter Tris-HCl (pH 9.0), 50 mmol/liter KCl, 2.0 mmol/liter $MgCl_2$, 0.1% Triton X-100, 0.01% gelatin, 200 μmol of each deoxynucleoside triphosphate/liter, 15 pmol each of the forward and reverse primers tagged with biotin at the 5′ end, and 1.5 U of AmpliTaq Gold (Perkin-Elmer). The mixture was incubated for 9 min at 94°C and for 40 cycles of 45 s at 45°C and 45 s at 72°C, with a final extension of 5 min at 72°C. Each experiment was performed with separate positive and negative PCR controls. The presence of HPV DNA was determined by hybridization of SPF_{10} amplimers to a mixture of general HPV probes recognizing a broad range of HPV genotypes in a microtiter plate format, as described previously (20).

(ii) HPV genotyping by reverse hybridization using the INNO-LiPA HPV genotyping system. A poly(dT) tail was enzymatically added to the 3′ end of each of 25 oligonucleotides specific for 25 types, namely, types 6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68/73, 70, and 74. The tailed probes were applied as horizontal lines to membrane strips (Labo Biomedical Products bv, Rijswijk, The Netherlands). The HPV genotyping assay was performed as described previously (20). In brief, equal volumes (10 μl each) of the biotinylated PCR products and denaturation solution (400 mmol/liter NaOH, 10 mmol/liter EDTA) were mixed in test troughs and incubated at room temperature for 5 min, after which 1 ml of prewarmed (37°C) hybridization solution (3× SSC [1× SSC is 0.15 mol/liter NaCl plus 0.015 mol/liter sodium citrate], 0.1% sodium dodecyl sulfate) was added. One strip was used per trough. Hybridization was performed for 1 h at 50 ± 0.5°C in a closed water bath with back-and-forth shaking. The strips were then washed twice with 1 ml of wash solution (3× SSC, 0.1% sodium dodecyl sulfate) at room temperature for 20 s and once at 50°C for 30 min. Following this stringent washing sequence, strips were rinsed twice with 1 ml of a standard rinse solution (20). Strips were then incubated on a rotating platform with an alkaline phosphatase-labeled streptavidin conjugate diluted in a standard conjugate solution at 20 to 25°C for 30 min, after which strips were washed twice with 1 ml of rinse solution and once with standard substrate buffer. Color development was initiated by the addition of 5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium to 1 ml of substrate buffer. After 30 min of incubation at room temperature, the color reaction was stopped by aspiration of the substrate buffer and the addition of distilled water. After drying, the strips were visually interpreted using a grid.

All discordant results were analyzed with the assumption that HPV26, HPV53, and HPV66 could be considered hrHPV genotypes or intermediate-risk HPV genotypes.

Statistical analysis. The accuracy, defined as the number of true positive samples plus the number of true negative samples/total number of patients, was used to compare HC2 and AMPLICOR. The kappa statistic was used to evaluate the agreement between HC2 and AMPLICOR.

The chi-square test was performed to compare proportions. Statistical analyses were performed using SAS software (SAS Institute Inc., NC).

RESULTS

Table 1 shows the results of the comparison between HC2 and AMPLICOR. As shown, 160/271 (59%) and 156/271

TABLE 1. Results of HC2 test and AMPLICOR HPV PCR test for the detection of high-risk HPV in 271 samples[a]

AMPLICOR HPV test result	No. of samples with HC2 result		Total no. of samples
	Negative	Positive	
Negative	95	20	115
Positive	16	140	156
Total	111	160	271

[a] The kappa statistic for comparison of the two tests is 0.73 ± 0.04.

(58%) samples were found to be positive for hrHPV by HC2 and AMPLICOR, respectively. Concordant results were obtained for 235 (86.7%) of the 271 samples (kappa statistic, 0.73 ± 0.04); only 36 (13.3%) of the 271 samples had discordant results between HC2 and AMPLICOR (Table 2). Of the discordant samples, 42% were mixed infections. Among the 36 discordant samples, the HC2 test detected hrHPV in 20 samples and AMPLICOR detected hrHPV in 16 samples.

HPV types 26, 53, and 66 as high-risk types. According to our reference for hrHPVs, when HPV53 and HPV66 are considered oncogenic, 8 of the 16 samples that were negative by HC2 and positive by AMPLICOR contained at least 1 of the 16 hrHPV genotypes and were considered truly positive by AMPLICOR and falsely negative by HC2. Thus, HPV18, HPV45, HPV53, HPV73, HPV18 plus HPV42, HPV42 plus HPV52, HPV52 plus HPV68, and HPV42 plus HPV45 plus HPV83 were detected in one sample each (Table 2).

According to our reference for hrHPVs, 14 of the 20 samples that were positive by HC2 and negative by AMPLICOR contained at least 1 of the 16 hrHPV genotypes and were considered truly positive by HC2 and falsely negative by AMPLICOR. Thus, HPV66 was detected in five samples, HPV53 was detected in three samples, HPV51 plus HPV39 was detected in two samples, and HPV62 plus HPV66, HPV66 plus HPV67, HPV66 plus HPV84, and HPV51 plus HPV62 plus HPV82 were detected in one sample each (Table 2). When considering HPV genotypes 53 and 66 as oncogenic and comparing HC2 and AMPLICOR results with the gold standard (INNO-LiPA HPV; Innogenetics), HC2 and AMPLICOR sensitivities were 95% and 91.4% (*P* is not significant), respectively, specificities were 94.5% and 92.7% (*P* is not significant), respectively, negative predictive values were 92.8% and 87.8% (*P* is not significant), respectively, and positive predictive values were 96.3% and 94.9% (*P* is not significant), respectively. Accuracies for HC2 and AMPLICOR were 94.8% and 91.9%, respectively (*P* is not significant).

HPV types 26, 53, and 66 as intermediate-risk types. According to our reference for hrHPVs, 7 of the 16 samples that were negative by HC2 and positive by AMPLICOR contained at least 1 of the 13 hrHPV genotypes and were considered falsely negative by HC2 and truly positive by AMPLICOR. Thus, HPV18, HPV45, HPV73, HPV18 plus HPV42, HPV42 plus HPV52, HPV52 plus HPV68, and HPV42 plus HPV45 plus HPV83 were detected in one sample each (Table 2).

According to our reference for hrHPVs, 3 of the 20 samples that were positive by HC2 and negative by AMPLICOR contained at least 1 of the 13 hrHPV genotypes and were considered truly positive by HC2 and falsely negative by AMPLICOR. Thus, HPV51 plus HPV39 was detected in two samples, and HPV51 plus HPV62 plus HPV82 was detected in one sample (Table 2).

TABLE 2. Overview of discordant cases

HPV genotype (*n*)	HC2 HPV assay result	AMPLICOR HPV assay result
HPV42 (2)	Positive	Negative
HPV53 (3)	Positive	Negative
HPV66 (5)	Positive	Negative
HPV70 (2)	Positive	Negative
HPV51 + HPV39 (2)	Positive	Negative
HPV62 + HPV66 (1)	Positive	Negative
HPV66 + HPV67 (1)	Positive	Negative
HPV66 + HPV84 (1)	Positive	Negative
HPV51 + HPV62 + HPV82 (1)	Positive	Negative
HPV54 + HPV61 + HPV70 + HPV81 (1)	Positive	Negative
Negative (1)	Positive	Negative
HPV18 (1)	Negative	Positive
HPV45 (1)	Negative	Positive
HPV53 (1)	Negative	Positive
HPV73 (1)	Negative	Positive
HPV83 (1)	Negative	Positive
HPV84 (1)	Negative	Positive
HPV18 + HPV42 (1)	Negative	Positive
HPV42 + HPV52 (1)	Negative	Positive
HPV52 + HPV68 (1)	Negative	Positive
HPV6 + HPV55 + HPV62 (1)	Negative	Positive
HPV42 + HPV45 + HPV83 (1)	Negative	Positive
Negative (5)	Negative	Positive

When considering HPV genotypes 26, 53, and 66 as not oncogenic and comparing HC2 and AMPLICOR results with the gold standard (INNO-LiPA HPV; Innogenetics), HC2 and AMPLICOR sensitivities were 94.7% and 98% (*P* is not significant), respectively, specificities were 85.8% and 93.3% (*P* is not significant), respectively, negative predictive values were 92.8% and 97.4% (*P* is not significant), respectively, and positive predictive values were 89.4% and 94.9% (*P* is not significant), respectively. Accuracies for HC2 and AMPLICOR were 95.9% and 90.8%, respectively ($P < 0.05$).

DISCUSSION

Sensitive and specific methods with high negative predictive values are needed to diagnose HPV infections associated with cervical lesions, particularly when HPV is used for triaging patients with ASCUS. The present study evaluates, in a prospective way, HC2 and AMPLICOR for detecting hrHPV in patients with ASCUS. The concordance between the HC2 and AMPLICOR assays was 235/271 samples (86.7%), with a kappa statistic of 0.73 ± 0.04, and hrHPV was found in 160/271 (59%) and 156/271 (58%) samples by HC2 and AMPLICOR, respectively. This concordance was similar to the 85.9% value reported by Poljak et al. (17), and the positivity of hrHPV detection for ASCUS samples was similar to the 63% value found by van Ham et al. (20).

Regarding the specificity of hrHPV detection, analysis of the discordant results with an independent assay (INNO-LiPA) as a reference test evidenced three main causes of discordances, namely, multiple infections (42%), the presence of genotypes 66 and 53 in 25% of the cases, and undetermined genotypes.

Although HPV53 and HPV66 are not included in the HC2 or AMPLICOR test, these genotypes are probably carcinogenic (14), and thus HPV53 and HPV66 were included in the direct comparison. Considering the diagnostic values of the assays, our study pointed out the difficulty in comparing the methods for genotypes classified as types 26, 53, and 66. When considering HPV genotypes 26, 53, and 66 as oncogenic and comparing HC2 and AMPLICOR results with our reference standard (INNO-LiPA), the accuracies for HC2 and AMPLICOR were 94.8% and 91.9%, respectively (P is not significant). These results can be explained by the cross-hybridization of HC2 in detecting types 53 and 66. Conversely, when considering HPV genotypes 26, 53, and 66 as not oncogenic and comparing HC2 and AMPLICOR results with our reference standard (INNO-LiPA), the accuracies of HC2 and AMPLICOR were 95.9% and 90.8%, respectively ($P < 0.05$).

Because of the large prevalence of multiple and persistent hrHPV infections found within specific lesions (42% of the discordant results in the present study), the lower specificity of the HC2 or AMPLICOR test can be considered an advantage. However, neither of the two assays was superior in detecting multiple infections (data not shown).

Regarding detection sensitivity, AMPLICOR is more sensitive than the HC2 hybridization assay (which may be compromised when the copy number of HPV DNA is low); the false-negative results with AMPLICOR were due mainly to mixed genotypes. The clinical importance of the higher analytical sensitivity of PCR-based HPV detection methods, such as AMPLICOR, than that of HC2 is debated. Some authors hypothesized that a high HPV viral load is indicative of viral persistence and disease development, while a low HPV viral load is associated with the clearance of an infection and even regression of cervical lesions (15).

Like HC2, the AMPLICOR HPV test is sensitive, specific, feasible, and easy to handle in routine but does not provide specific genotype information. The performance of the AMPLICOR HPV test for prediction of cervical intraepithelial neoplasia was recently reported (13). AMPLICOR was assessed for women with abnormal cervical smears, with colposcopic biopsy and liquid-based cytology as the reference standards. AMPLICOR provides an alternative to rather than an advance over HC2, which might drive down the price for HPV testing by providing a competitor.

Many studies reported a strong concordance between the type of HPV found in the baseline smear before the development of cervical carcinoma and the type of HPV found in the biopsy specimen of the invasive cancer, indicating that neoplasia is associated with HPV type-specific persistence (2, 3, 5–7, 12). Future management of patients with neoplasia will probably include not only HPV detection but HPV genotyping as well.

REFERENCES

1. **ALTS Group.** 2000. Human papillomavirus testing for triage of women with cytologic evidence of low-grade squamous intraepithelial lesions: baseline data from a randomized trial. J. Natl. Cancer Inst. **92:**397–402.
2. **Castle, P. E., M. Schiffman, R. Herrero, A. Hildesheim, A. C. Rodriguez, M. C. Bratti, M. E. Sherman, S. Wacholder, R. Tarone, and R. D. Burk.** 2005. A prospective study of age trends in cervical human papillomavirus acquisition and persistence in Guanacaste, Costa Rica. J. Infect. Dis. **191:** 1808–1816.
3. **Castle, P. E., D. Solomon, M. Schiffman, and C. M. Wheeler.** 2005. Human papillomavirus type 16 infections and 2-year absolute risk of cervical precancer in women with equivocal or mild cytologic abnormalities. J. Natl. Cancer Inst. **97:**1066–1071.
4. **Castle, P. E., C. M. Wheeler, D. Solomon, M. Schiffman, and C. L. Peyton.** 2004. Interlaboratory reliability of Hybrid Capture 2. Am. J. Clin. Pathol. **122:**238–245.
5. **Clifford, G. M., S. Gallus, R. Herrero, N. Munoz, P. J. Snijders, S. Vaccarella, P. T. Anh, C. Ferreccio, N. T. Hieu, E. Matos, M. Molano, R. Rajkumar, G. Ronco, S. de Sanjose, H. R. Shin, S. Sukvirach, J. O. Thomas, S. Tunsakul, C. J. Meijer, and S. Franceschi.** 2005. Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. Lancet **366:**991–998.
6. **Clifford, G. M., R. K. Rana, S. Franceschi, J. S. Smith, G. Gough, and J. M. Pimenta.** 2005. Human papillomavirus genotype distribution in low-grade cervical lesions: comparison by geographic region and with cervical cancer. Cancer Epidemiol. Biomarkers Prev. **14:**1157–1164.
7. **Clifford, G. M., J. S. Smith, T. Aguado, and S. Franceschi.** 2003. Comparison of HPV type distribution in high-grade cervical lesions and cervical cancer: a meta-analysis. Br. J. Cancer **89:**101–105.
8. **Davies, P., J. Kornegay, and T. Iftner.** 2001. Current methods of testing for human papillomavirus. Best Pract. Res. Clin. Obstet. Gynaecol. **15:**677–700.
9. **Gravitt, P. E., C. L. Peyton, T. Q. Alessi, C. M. Wheeler, F. Coutlee, A. Hildesheim, M. H. Schiffman, D. R. Scott, and R. J. Apple.** 2000. Improved amplification of genital human papillomaviruses. J. Clin. Microbiol. **38:**357–361.
10. **Kulmala, S. M., S. Syrjanen, I. Shabalova, N. Petrovichev, V. Kozachenko, J. Podistov, O. Ivanchenko, S. Zakharenko, R. Nerovjna, L. Kljukina, M. Branovskaja, V. Grunberga, A. Juschenko, P. Tosi, R. Santopietro, and K. Syrjanen.** 2004. Human papillomavirus testing with the hybrid capture 2 assay and PCR as screening tools. J. Clin. Microbiol. **42:**2470–2475.
11. **Lörincz, A., and J. Anthony.** 2001. Advances in HPV detection by hybrid capture. Papillomavir. Rep. **12:**145–154.
12. **Mendez, F., N. Munoz, H. Posso, M. Molano, V. Moreno, A. J. van den Brule, M. Ronderos, C. Meijer, and A. Munoz.** 2005. Cervical coinfection with human papillomavirus (HPV) types and possible implications for the prevention of cervical cancer by HPV vaccines. J. Infect. Dis. **192:**1158–1165.
13. **Monsonego, J., J. M. Bohbot, G. Pollini, C. Krawec, C. Vincent, I. Merignargues, F. Haroun, P. Sednaoui, L. Monfort, R. Dachez, and K. Syrjanen.** 2005. Performance of the Roche AMPLICOR human papillomavirus (HPV) test in prediction of cervical intraepithelial neoplasia (CIN) in women with abnormal Pap smear. Gynecol. Oncol. **99:**160–168.
14. **Munoz, N., F. X. Bosch, S. de Sanjose, R. Herrero, X. Castellsague, K. V. Shah, P. J. Snijders, and C. J. Meijer.** 2003. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N. Engl. J. Med. **348:**518–527.
15. **Perrons, C., R. Jelley, B. Kleter, W. Quint, and N. Brink.** 2005. Detection of persistent high risk human papillomavirus infections with hybrid capture II and SPF10/LiPA. J. Clin. Virol. **32:**278–285.
16. **Peyton, C. L., M. Schiffman, A. T. Lorincz, W. C. Hunt, I. Mielzynska, C. Bratti, S. Eaton, A. Hildesheim, L. A. Morera, A. C. Rodriguez, R. Herrero, M. E. Sherman, and C. M. Wheeler.** 1998. Comparison of PCR- and hybrid capture-based human papillomavirus detection systems using multiple cervical specimen collection strategies. J. Clin. Microbiol. **36:**3248–3254.
17. **Poljak, M., K. Fujs, K. Seme, B. J. Kocjan, and E. Vrtacnik-Bokal.** 2005. Retrospective and prospective evaluation of the Amplicor HPV test for detection of 13 high-risk human papillomavirus genotypes on 862 clinical samples. Acta Dermatovenerol. Alp. Panonica Adriat. **14:**147–152.
18. **Smith, R. A., V. Cokkinides, and H. J. Eyre.** 2003. American Cancer Society guidelines for the early detection of cancer. CA Cancer J. Clin. **53:**27–43.
19. **Snijders, P. J., A. J. van den Brule, and C. J. Meijer.** 2003. The clinical relevance of human papillomavirus testing: relationship between analytical and clinical sensitivity. J. Pathol. **201:**1–6.
20. **van Ham, M. A., J. M. Bakkers, G. K. Harbers, W. G. Quint, L. F. Massuger, and W. J. Melchers.** 2005. Comparison of two commercial assays for detection of human papillomavirus (HPV) in cervical scrape specimens: validation of the Roche AMPLICOR HPV test as a means to screen for HPV genotypes associated with a higher risk of cervical disorders. J. Clin. Microbiol. **43:**2662–2667.
21. **Walboomers, J. M., M. V. Jacobs, M. M. Manos, F. X. Bosch, J. A. Kummer, K. V. Shah, P. J. Snijders, J. Peto, C. J. Meijer, and N. Munoz.** 1999. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J. Pathol. **189:**12–19.
22. **Wright, T. C. J., J. Cox, L. Massad, L. Twiggs, E. Wilkinson, et al.** 2002. Consensus guidelines for the management of women with cervical cytological abnormalities. JAMA **287:**2120–2129.