

Performance of the Roche AMPLICOR® Human papillomavirus (HPV) test in prediction of cervical intraepithelial neoplasia (CIN) in women with abnormal PAP smear

Joseph Monsonogo^{a,*}, Jean Marc Bohbot^a, Giuseppe Pollini^a, Claude Krawec^a, Catherine Vincent^a, Isabelle Merignargues^a, Fatima Haroun^a, Patrice Sednaoui^a, Laura Monfort^a, Roger Dachez^a, Kari Syrjänen^b

^aInstitut Alfred Fournier, 25 Boulevard St. Jacques, 75014 Paris, France

^bDepartment of Oncology and Radiotherapy, Turku University Central Hospital, Kiinamyllynkatu 4-8, FIN-20521 Turku, Finland

Received 28 February 2005

Available online 14 July 2005

Abstract

Objectives. To assess the performance of a novel PCR-based assay (Roche AMPLICOR® HPV test) in detection of cervical pathology as a part of management for abnormal PAP smear (MAPS) and in women participating in cervical cancer screening.

Study design. Altogether, 504 women comprising 270 patients referred for colposcopy due to an abnormal Pap smear and another 234 women participating in cervical cancer screening (tested for comparison) were analyzed for oncogenic (HR) Human papillomavirus (HPV) types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 using the Roche AMPLICOR® HPV test in cervical samples collected in PreservCyt® liquid media. Colposcopic biopsy and/or LEEP cone biopsy was used as the gold standard in the triage group, while liquid-based cytology (LBC) was the reference test in the screening group.

Results. The prevalence of HPV was significantly higher in the MAPS group (65.9%) than in the screening group (31.2%) ($P = 0.0001$). There was a poor concordance between the referral PAP and the current LBC, being only moderate in the screening series, ICC (weighted kappa) = 0.291 (95%CI 0.070–0.459) ($P = 0.007$), and almost poor in the MAPS Series, with ICC = 0.217 (95%CI 0.04–0.384) ($P = 0.023$). AMPLICOR® HPV positivity increased linearly with the increasing grade of cervical lesions. In detecting high-grade (CIN2–3), colposcopy was the most sensitive test (96.5%), very similar to AMPLICOR (95.2%) ($P = 0.731$), while LBC with HSIL cutoff was by far the most specific test (99.5%) and showed the highest PPV (96.1%). NPV of colposcopy (97.2%) and AMPLICOR (96.7%) were similar ($P = 0.839$). Together with abnormal colposcopy and HSIL cytology, the AMPLICOR® HPV test is a powerful independent predictor of high-grade CIN2–3, and as such suitable to replace cervical cytology in management of women with abnormal PAP test (MAPS).

Conclusions. The Roche AMPLICOR® HPV test is comparable to other HPV tests (HCII, PCR) in detecting CIN in MAPS. However, more data are clearly needed on the performance of AMPLICOR test in management of abnormal PAP and particularly as a screening tool. © 2005 Elsevier Inc. All rights reserved.

Keywords: HPV; AMPLICOR test; Management; Abnormal PAP; Colposcopy; Liquid-based cytology; Test performance; CIN

Introduction

Cervical cancer has an uneven geographic distribution, with the vast majority of cases being confined to regions

where the resources to combat the disease are the most meager, i.e., in the developing countries [1–4]. There is no argument that the declining trends in incidence and mortality rates witnessed in the developed countries during the past four decades are mainly attributable to the implementation of organized screening programs based on cervical Pap smear [5–8]. The best examples are the Nordic countries, where organized screening has resulted in up to

* Corresponding author. Colposcopy Unit, Institut Fournier, 174 rue de Courcelles, 75017 Paris, France. Fax: +33 1 47 66 74 70.

E-mail address: jm@eurogin.com (J. Monsonogo).

80% reduction in cervical cancer incidence since the early 1960s [6,9,10].

Unfortunately, these highly effective organized screening programs exist in few countries only, and the prospects for effective PAP smear screening in the majority of the developing countries seem gloomy, if not entirely pessimistic, in the foreseeable future [6–8,11,12]. This fact has been well recognized among the scientific community, emphasizing the necessity to find other solutions to cope with this increasing problem [5–8,11,12]. A variety of optional screening tools have been recently introduced to substitute the PAP test [6–8,11,12]. These include: visual inspection with acetic acid (VIA) or Lugol's iodine (VILI) [13,14], cervicography [15], speculscopy [16], screening colposcopy [17], liquid-based (LBC), and automated cytology [18,19], all under rigorous testing in different settings [13–20].

Testing for Human papillomavirus (HPV) by different molecular tools (Hybrid Capture, PCR) has been proposed as an adjunct or independent screening tool, with several potential advantages [7,8,11–13,21–25]. Testing for the etiological agent of cervical cancer [3,4] offers an opportunity to detect the women at increased risk of cervical cancer at the stage of latent and subclinical HPV infection, preceding (by several months to years) the clinical stages (SIL, CIN) detectable by the PAP test, which makes cervical cancer unique among all human malignancies [3,4,11,12,22,23,25]. Apart from the primary screening, management of abnormal PAP (triage) is another potential area of usage for HPV testing, extensively discussed in the recent literature [4,8,11,12,15,26–30].

High-throughput assays suitable for large-scale testing are based on two different technologies: (1) hybridization-based assays (e.g., Hybrid Capture II) and (2) tests utilizing PCR-based technology. The advantages and disadvantages of these two basically different assays have been extensively discussed [3,4,8,11,12,21–23], and several recent studies are available comparing the performance of HCII and PCR techniques in both triage and in the primary screening [31–36]. Recently, a novel qualitative HPV test was introduced, known as the AMPLICOR® Human Papilloma Virus (HPV) Test (Roche Molecular Systems). This new test utilizes amplification of target DNA by PCR and nucleic acid hybridization for the detection of high-risk (HR) HPV DNA genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 in cervical cells collected into an LBC media [37–39].

Until now, no published studies are available on the use of AMPLICOR HPV test in clinical practice (triage or screening), despite a series of recent congress abstracts, where the test was validated for use in two different sample collection media [37–47]. We recently designed a study where this new assay was tested in management of women with abnormal PAP smears (MAPS). The present communication reports the results of this study, where performance indicators of AMPLICOR were compared with those of

LBC (ThinPrep) and colposcopy, in detecting CIN (CIN1 and CIN2–3) as the outcome variable in a series of women examined for abnormal PAP smears.

Materials and methods

Patients

In the present study, we examined 270 women referred for colposcopic examination due to an abnormal PAP smear (MAPS Series), and for comparison, another series of 234 women participating in opportunistic cervical cancer screening in Paris, France (Screening Series). Both series were examined in the same clinic (Institute Alfred Fournier, IAF), during November 2004, by 2 colposcopists (JM, GP).

The MAPS Series comprises a total of 270 consecutive women referred for colposcopy due to an abnormal PAP smear. The mean age of the women was 35.0 years (SD 11.2, range 18–75, median 33 years). The proportion of women below 35 years of age was 58.5%. These women had a PAP smear taken in different clinics in Paris, and referred for colposcopic examination to IAF (and two private colposcopy clinics). These women had a new PAP smear taken and all were examined by colposcopy and cervical biopsy or treated by LEEP cone.

In addition to the recorded screening history, the 234 women in the Screening Series were examined by cervical cytology, but they were not subjected to colposcopy or cervical biopsy. The mean age of these patients was 38.8 years (SD 13.8, range 19–79, median 35 years). Because of the lack of a reference test (gold standard), no test performance indicators were calculated for these women, the main focus being in assessment of the HPV prevalence among this screening population.

Methods

Cytology

All women in the MAPS Series had a previous PAP smear taken within 3–4 months prior to their enrollment in the study (i.e., the referral PAP), performed by community physicians. The mean referral time from the first PAP smear to the clinical examination was 2.9 ± 2.2 (SD) months. These baseline smears were examined by cytologists in several different laboratories in Paris, and were not available for re-examination by the authors. The smears were classified according to the 2001 Bethesda system (TBS 2001), and the original diagnoses were used as the baseline referral PAP smear diagnoses.

From women in the Screening Series, only the information about their screening history was recorded, including the time lapsed since the last PAP smear as well as whether the previous smear was normal or abnormal. In exceptional cases, more accurate information was available on the previous PAP smear result.

In both the MAPS and Screening Series, a new cervical smear was taken at the clinical visit from every woman. Cervical samples for Liquid Based Cytology (LBC) were collected by a specially designed sampling device, which was rinsed into an LBC media PreservCyt® (ThinPrep liquid PAP vial) (Cytoc Corporation, US), and prepared for ThinPrep specimens, following the manufacturer's recommendations. This media is also validated for use with the AMPLICOR HPV Test [37–39].

Colposcopy

After sampling for LBC and HPV DNA testing, colposcopic examination of the cervix, vagina, and vulva was performed for all patients of the Triage Series by the different colposcopists, using a jointly agreed protocol. Lesions in the transformation zone (TZ) were assessed by applying 5% acetic acid and iodine solution, under 8× to 12× magnification. If colposcopy proved unsatisfactory, further exploration of the endocervix was systematically carried out under 20× magnification using a Koogan speculum [48]. The international (IFCPC) nomenclature [49] was used to classify the colposcopic patterns as: normal, abnormal TZ (ATZ) with minor changes (with or without features of HPV infection), suggesting low grade CIN (CIN1), ATZ with major changes suggesting CIN2–3, and cancer. For statistical analysis, colposcopic results were dichotomized as either (a) normal or (b) abnormal.

Biopsy procedures

All 270 women in the MAPS Series underwent colposcopic examination. Biopsy was not taken from 59 women because of normal colposcopic appearance, and considered as having no cervical pathology. LEEP cone biopsy was performed in cases with (a) PAP test showing HSIL and ATZ in colposcopy, (b) regardless of the PAP test result, if the ATZ was large ($\geq 50\%$ of TZ area), (c) an endocervical lesion and unsatisfactory colposcopy, or (d) ATZ and a squamo-columnar junction localized more than 3 mm within the endocervix. Altogether, 42 women underwent treatment by LEEP cone, while the rest ($n = 169$) had a directed punch biopsy taken.

Histology

All biopsies were examined in one pathology laboratory in Paris (Laboratoire Claude-Levy) and reported by one pathologist (RD). Because of their known poor reproducibility, all CIN1 lesions were re-examined by an independent second pathologist (KS), who also reviewed all discrepant cases, where PAP smear and biopsy were discordant by more than two grades, e.g., normal PAP test and CIN2–3 histology, or vice versa. Result of this review was used as the final diagnosis. Histological assessments were made as blinded by the HPV DNA status. In classifying the biopsies, the CIN terminology was adopted [3]. In calculating the performance characteristics of cytology, colposcopy, and AMPLICOR HPV test, the consensus diagnoses were used

as the gold standard, and in statistical calculations, two cutoff values were used: CIN1 and CIN2–3.

HPV DNA detection

In this study, HPV testing was done using the AMPLICOR® HPV test (Roche Molecular Systems), strictly following the manufacturer's instructions.

Specimen collection, transport, and storage. Specimens were collected into PreservCyt® LBC media (ThinPrep liquid PAP vial; Cytoc Corporation, US), validated for use with the AMPLICOR HPV Test, following the manufacturer's instructions [37–39]. Specimens collected in PreservCyt medium were transported to laboratory at 2–30°C. Before analysis, specimens may be stored at room temperature for up to 21 days or at 2–8°C for up to 8 weeks.

PCR amplification. In AMPLICOR HPV Test, a pool of HPV primers is designed to amplify HPV DNA from 13 high-risk genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) [40,41]. Capture probe sequences are located in 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 [42,43]. AmpliTaq GOLD® DNA Polymerase extends the annealed primers along the target templates to produce an approximately 165-base pair double-stranded HPV target DNA molecule or a 268-base pair β -globin DNA amplicon [41–47].

Hybridization reaction. Following 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 amplicon are hybridized to the oligonucleotide probes bound to the wells of the MWP. This hybridization of amplicon to the probes increases the overall specificity of the test [41–47].

Detection reaction. 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 Avidin–Horseradish Peroxidase Conjugate binds to the biotin-labeled amplicon hybridized to the oligonucleotide probes 2 (HPV or β -globin) bound to the MWP. According to the manufacturer's specification, AMPLICOR HPV test could 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 greater than 95%. All genotypes are detected with a 100% positivity rate at 480 copies/ml.

Statistical analysis

Statistical analyses were performed using the SPSS® and STATA software packages (SPSS for Windows, version 12.0.1. and STATA/SE 8.2). Frequency tables were analyzed

using the Chi-square test, and the likelihood ratio (LR) statistics or Fisher’s exact test (where appropriate) were used to assess the correlation between the categorical variables. OR and 95% confidence intervals (95%CI) were calculated where appropriate. Differences in the means of continuous variables between the groups were analyzed using non-parametric tests (Mann–Whitney). Performance indicators of cytology, colposcopy, and HPV testing in detection of the outcome variables (CIN1 or CIN2–3 in the MAPS Series and ASC-H, LSIL, HSIL in the Screening Series) were calculated using the conventional contingency tables to calculate sensitivity, specificity, positive (PPV) and negative predictive value (NPV), with 95%CI based on the *F* distribution ($\pm 1.96 \times SE$). The agreement (reproducibility) of cytological diagnosis was controlled by comparing the diagnosis of the referral PAP smear with the current LBC cytology, using the intra-class correlation coefficient (ICC), which is equivalent to weighted kappa [50]. Regular Cohen’s kappa was not computable, due to the different diagnostic categories in the two data sets. To run the ICC test, we used the parallel model, with the two-way random effects option, and calculated the ICC values using the consistency requirement [51]. Logistic regression models were used to analyze the power of different variables as predictors of the outcome variables (CIN, SIL) both in univariate (crude ORs and 95%CI) and multivariate (adjusted ORs and 95%CI) analyses, using the stepwise backward approach and LR (likelihood ratio) statistic for removal testing ($P = 0.10$ probability for stepwise removal, and $P = 0.05$ probability for stepwise entry). In all tests, the values $P < 0.05$ were regarded statistically significant.

Results

The prevalence of HPV was significantly higher in the MAPS group (65.9%) than in the screening group (31.2%) ($P = 0.0001$). OR for being HPV positive in a MAPS patient was 4.26 (95%CI 2.936–6.202), as compared with the screening group. HPV prevalence was significantly higher among women below 35 years of age (62.8%) as compared with those beyond that age (33.9%) ($P = 0.0001$) (OR 3.29, 95%CI 2.27–4.75). The same figures for women under and beyond age 30 were 64.1% and 40.8%, respectively (OR 2.59, 95%CI 1.79–3.76). The number of pregnancies and deliveries were not significantly related to HPV detection, $P = 0.459$ and $P = 0.225$, respectively. Having a partner or not did not contribute to HPV prevalence, 54.6% and 50.0%, respectively ($n = 381$) ($P = 0.826$).

There was a poor concordance between the referral PAP and the current LBC, being only moderate in the screening series, ICC (weighted kappa) = 0.291 (95%CI 0.070–0.459) ($P = 0.007$), and almost poor in the MAPS Series, with ICC = 0.217 (95%CI 0.04–0.384) ($P = 0.023$). Referral PAP smear was significantly (Chi-square,

Fisher’s exact test $P = 0.0001$) associated with subsequent HPV detection, as shown in Table 1. HPV DNA prevalence increased in parallel with the increasing atypia of the referral PAP smear from 30% in cases with normal smear up to 82% among those with HSIL PAP test at baseline ($P = 0.0001$ for linear trend).

Similarly, the findings on colposcopy ($n = 260$) were also significantly related to HPV detection ($P = 0.0001$), which increased from 36.4% among those with normal colposcopy, to 75.3% in minor, 75.0% in borderline, and 88.1% in women with major abnormality. OR for being HPV positive was 6.26 (95%CI 3.497–11.194) among those with abnormal colposcopy as compared to women with normal colposcopy ($P = 0.0001$) (data not shown in tables).

There was no difference in the referral time calculated from the referral PAP smear to the clinical visit, between HPV-positive and HPV-negative women; 2.8 and 3.2 months, respectively ($P = 0.164$ Mann–Whitney). The results of the current LBC cytology are related to HPV detection by AMPLICOR in Table 2. There is a linear increase of HPV prevalence in parallel with the increasing cytological abnormality, up to 92.6% among women with HSIL cytology ($P = 0.0001$ for linear trend).

Table 3 summarizes the HPV detection rates in different grades of cervical pathology. HPV prevalence increases in parallel with the increasing grade of the lesions from 50% with no cervical pathology (normal and metaplasia) up to 96.6% in CIN2–3 and 100% in invasive SCC.

Table 4 shows the relationship between cervical cytology and colposcopic patterns. The proportion of abnormal colposcopy is directly related to severity of PAP smear abnormality ($P = 0.0001$), reaching 96% among women with HSIL smear. Indeed, OR for abnormal colposcopy was 9.42 (95%CI 5.011–17.708) for those with abnormal PAP test (any abnormality), as compared with women who had normal PAP test. This OR increased to 11.39 (95%CI 1.513–85.808) for those who had HSIL PAP smear.

Table 5 summarizes the performance indicators of cervical cytology, colposcopy, and HPV test in detecting cervical pathology, using CIN1 and CIN2–3 cutoffs. The most sensitive test in detecting CIN1 was colposcopy,

Table 1
Referral PAP smear related to subsequent HPV DNA detection

Referral PAP smear	HPV test		Total
	Positive	Negative	
Normal	62 (30.4%)	142 (69.6%)	204 (100.0%)
ASC-US	70 (60.9%)	45 (39.1%)	115 (100.0%)
ASC-H	5 (62.5%)	3 (37.5%)	8 (100.0%)
LSIL	79 (66.4%)	40 (33.6%)	119 (100.0%)
HSIL	23 (82.1%)	5 (17.9%)	28 (100.0%)
Glandular atypia	3 (42.9%)	4 (57.1%)	7 (100.0%)
Total	242 (50.3%)	239 (49.7%)	481 (100.0%)

Chi-square, Fisher’s exact test $P = 0.0001$ (also for linear trend).

Table 2
Results of cervical cytology related to HPV DNA detection

Current LBC diagnosis*	HPV test		Total
	Positive	Negative	
Inadequate	2 (100.0%)	0 (0.0%)	2 (100.0%)
Normal	104 (31.7%)	224 (68.3%)	328 (100.0%)
ASC-US	50 (72.5%)	19 (27.5%)	69 (100.0%)
ASC-H	6 (85.7%)	1 (14.3%)	7 (100.0%)
LSIL	61 (89.7%)	7 (10.3%)	68 (100.0%)
HSIL	25 (92.6%)	2 (7.4%)	27 (100.0%)
Total	248 (49.5%)	253 (50.5%)	501 (100.0%)

Chi-square, Fisher's exact test $P = 0.0001$.

* Consensus diagnosis.

followed by AMPLICOR HPV Test, while the specificity of cervical cytology (with all cutoffs) was superior to the other methods. The same was true with the PPV, whereas the highest NPV was ascribed to colposcopy and AMPLICOR. In detecting high-grade (CIN2–3) lesions, colposcopy was the most sensitive test (96.5%), very similar to AMPLICOR (95.2%) ($P = 0.731$), while cervical cytology with HSIL cutoff was by far the most specific test (99.5%) and showed the highest PPV (96.1%). The NPV of colposcopy (97.2%) and AMPLICOR (96.7%) were very similar ($P = 0.839$).

The performance of AMPLICOR HPV test in detecting abnormal cytology in the MAPS Series is summarized in Table 6. Indeed, OR for positive HPV test is very similar at all PAP smear cutoff levels, around 7.4, being highly significant ($P = 0.0001$). The highest sensitivity and NPV are found at HSIL cutoff, while specificity and PPV are higher with lower PAP smear cutoff (LSIL, ASC-H).

Discussion

In the present study, we tested the performance of a novel HPV test (the Roche AMPLICOR® HPV test)

Table 3
HPV prevalence related to the grade of cervical lesions

Cervical biopsy or cone histology*	HPV test		Total
	Positive	Negative	
Biopsy not taken	21 (36.2%)	38 (63.8%)	59 (100.0%)
Normal and squamous metaplasia	32 (49.3%)	33 (50.7%)	65 (100.0%)
CIN1 ^a	61 (80.2%)	15 (19.8%)	76 (100.0%)
CIN2–3	57 (96.6%)	2 (3.4%)	59 (100.0%)
Squamous cell cancer**	1 (100.0%)	0 (0.0%)	1 (100.0%)
VAIN1	4 (66.6%)	2 (33.4%)	6 (100.0%)
VAIN2–3	2 (66.6%)	1 (33.4%)	3 (100.0%)
Total	178 (66.4%)	90 (33.6%)	268 (100.0%)

Chi-square test, Fisher's exact test $P = 0.0001$.

^a One case of cervical condyloma included.

* Consensus diagnosis.

** A micro-invasive SCC.

Table 4
Colposcopic findings related to results of cervical cytology

Current LBC diagnosis*	Colposcopic pattern abnormal**		Total
	Yes	No	
Inadequate	2 (100.0%)	0 (0.0%)	2 (100.0%)
Normal	47 (44.3%)	59 (55.7%)	106 (100.0%)
ASC-US	48 (80.0%)	12 (20.0%)	60 (100.0%)
ASC-H	6 (100.0%)	0 (0.0%)	6 (100.0%)
LSIL	55 (93.2%)	4 (6.8%)	59 (100.0%)
HSIL	24 (96.0%)	1 (4.0%)	25 (100.0%)
Total	182 (70.5%)	76 (29.5%)	258 (100.0%)

Chi-square, Fisher's exact test $P = 0.0001$.

* Consensus diagnosis.

** Includes minor (TA1, TA1b) and major (TA2) changes.

[37,38,45–47] both in management of women with abnormal PAP test (MAPS) and in a setting of an opportunistic screening for cervical cancer. Colposcopic or cone biopsy was used as the gold standard in the triage group, while LBC cytology was the reference test in the screening series, against which the performance indicators of the new test were calculated. The rationale of this study design is to avoid the verification bias in both series, while all women in the MAPS group were examined by colposcopy (and biopsy), while all women in the screening group were verified by LBC only [22,25,27]. This is important to provide unbiased figures for test performance in these two different settings [4,8,15,21–23,25–30].

Not unexpectedly, the prevalence of HPV was significantly higher in the MAPS group (65.9%) than in the screening group (31.2%) ($P = 0.0001$). This has been confirmed in most of the previous studies reporting HPV prevalence in different cohorts [3,4,15,20,24,25,28,29,31,33–35,48,52,53]. Similarly, HPV prevalence was clearly age-dependent, being significantly higher among younger women (30 or 35 years). This age dependence was more marked in the screening series (OR 4.19, 95%CI 2.28–7.72) than in the MAPS group (OR 2.94, 95%CI 1.74–4.94) ($P = 0.0001$, between the two groups). This should have implications in selecting the appropriate target age groups for screening by HPV tests, as recently discussed [20–25,34,35]. On the other hand, this age distinction seems to be less important in MAPS, where HPV prevalence is high in both younger (75.9%) and older (51.8%) women, implicating that testing for oncogenic HPV might be justified in all age groups. Apart from the age, none of the other recorded variables (number of pregnancies, deliveries, having a sexual partner, previous PAP smear screening history) was of any predictive value for HPV detection in these two series. This is not unexpected, however, because much more detailed recording of the epidemiological data is usually needed to disclose the risk factors of HPV [54], which was not even attempted in the present study.

In the management of women with abnormal PAP smears, the diagnostic set-up usually starts from an abnormal PAP test leading to referral for colposcopy, taking punch biopsy and

Table 5

The performance characteristics of PAP test, colposcopy, and AMPLICOR HPV test in detecting cervical pathology

Diagnostic test	OR (95%)	Outcome measure			
		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
<i>CIN 1*</i>					
PAP: LSIL*	10.75 (5.60–20.66)	55.0 (46.5–63.6)	89.8 (84.7–94.9)	83.5 (75.6–91.4)	67.9 (61.2–74.8)
PAP: ASC-H*	12.04 (6.34–22.87)	59.7 (51.2–68.2)	89.0 (83.8–94.3)	83.7 (76.1–91.2)	70.1 (63.3–76.9)
PAP: HSIL*	32.69 (4.35–245.22)	19.4 (12.6–26.2)	99.3 (97.8–100.0)	96.2 (88.8–100.0)	56.7 (50.4–62.9)
Colposcopic pattern [#]	8.76 (4.31–17.80)	90.8 (85.7–96.0)	46.9 (38.3–55.5)	61.2 (54.1–63.4)	84.7 (76.4–93.0)
AMPLICOR HPV test	10.16 (5.31–19.41)	89.2 (83.9–94.6)	55.1 (46.8–63.4)	65.2 (58.2–72.2)	84.4 (76.9–91.9)
<i>CIN2–3*</i>					
PAP: LSIL*	7.10 (3.80–13.23)	66.1 (54.3–77.9)	78.4 (72.8–84.1)	48.2 (37.6–58.9)	88.4 (83.7–93.1)
PAP: ASC-H*	8.84 (4.63–16.87)	72.6 (61.5–83.7)	76.9 (71.2–82.7)	48.9 (38.7–59.1)	90.2 (85.8–94.6)
PAP: HSIL*	137.2 (18.02–1043.6)	40.3 (28.1–52.5)	99.5 (98.6–100.0)	96.1 (88.8–100.0)	84.5 (80.0–89.2)
Colposcopic pattern [#]	15.65 (3.70–66.12)	96.5 (91.7–100.0)	36.3 (29.5–43.0)	30.9 (24.1–37.7)	97.2 (93.4–100.0)
AMPLICOR HPV test	14.75 (4.47–48.57)	95.2 (89.9–100.0)	42.4 (35.7–49.2)	33.7 (26.8–40.7)	96.7 (93.0–100.0)

* Consensus diagnosis.

[#] Abnormal vs. normal.

ending up with the histopathological examination of the biopsy or cone [26]. This sequence of diagnostic steps can be complemented by adding HPV testing, exactly as done in the present study. In this doing, we can analyze the performance indicators of, e.g., referral cytology in predicting three outcome measures in these women, which are: (1) abnormal colposcopy, (2) significant cervical pathology, and (3) detection of oncogenic HPV. This type of analysis was precluded in the screening series, however, where detailed data on the referral smear (previous PAP test) was not available, and HPV testing and LBC were the only confirmatory tests performed.

When this analysis was done for the women in the MAPS Series, we noticed that referral PAP smear atypia showed a linear association to HPV prevalence (Table 1). On the other hand, the value of referral PAP in predicting positive colposcopy was not particularly good with LSIL cutoff (OR 1.96, 95% CI 1.14–3.37) ($P = 0.015$), and of no significance with the HSIL cutoff; OR 1.79 (95% CI 0.70–4.59) ($P = 0.289$). The figures are completely different for current LBC in detecting positive colposcopy, with all (ASC-H, LSIL, and HSIL) cutoff values: OR 12.44 (95% CI 4.80–32.25), OR 10.81 (95% CI 4.19–28.25),

and OR 11.39 (95% CI 1.51–85.80), respectively (data not in tables). The specificities were 93.4%, 93.4%, and 98.7%, respectively, for ASC-H, LSIL, and HSIL cutoffs. Thus, current LBC assay is a highly specific tool in predicting positive colposcopy (Table 4), which is consonant with the previous experience [18,20,25,27,55]. However, abnormal colposcopy could be equally well predicted by the AMPLICOR HPV test, as shown by the fact that OR for having abnormal colposcopy was 6.26 (95% CI 3.497–11.194) among HPV-positive women as compared to HPV-negative women ($P = 0.0001$).

While assessing the performance indicators of the referral PAP test in detecting cervical pathology, the results were poor (data not in tables). The only significant OR was obtained for referral PAP at HSIL cutoff in detecting CIN2–3 pathology (OR 5.10, 95% CI 2.43–10.70), with SE 14.3%, SP 92.2%, PPV 54.3%, and NPV 81.1%. This indicates a poor agreement between the referral PAP and the current LBC as measured by weighted kappa (ICC). This emphasizes the importance of controlling the PAP test prior to making colposcopy. This inevitably increases the costs of the triage, while an inadequately performing test must be

Table 6

The performance indicators of AMPLICOR HPV test in predicting abnormal cervical cytology in the MAPS Series

Diagnostic test	OR (95%)	Outcome measure			
		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
<i>ASC-US*</i>					
AMPLICOR HPV test	7.17 (4.08–16.62)	83.3 (77.5–89.2)	58.9 (49.8–68.0)	73.9 (67.4–80.4)	71.7 (62.5–80.9)
<i>ASC-H*</i>					
AMPLICOR HPV test	7.48 (3.64–15.38)	89.4 (83.1–95.6)	47.1 (39.7–54.5)	47.7 (40.3–55.1)	89.1 (82.8–95.5)
<i>LSIL*</i>					
AMPLICOR HPV test	7.34 (3.47–15.53)	89.7 (83.3–96.1)	45.9 (38.6–53.1)	44.3 (37.0–51.7)	90.2 (84.1–96.3)
<i>HSIL*</i>					
AMPLICOR HPV test	7.45 (1.72–32.20)	92.6 (82.7–100)	37.3 (31.2–43.4)	14.2 (9.0–19.4)	97.8 (94.8–100.0)

* Consensus diagnosis.

controlled by a second test (LBC in this case). This is also one of the arguments favoring the use of HPV testing (instead of cytology) as the triage tool, particularly in settings where PAP smear cytology is not optimally performed [4,8,15,26–30,48,52,53,55]. Indeed, the present data (Table 2) indicate that AMPLICOR HPV test showed a linear increase of HPV prevalence in parallel with the increasing LBC abnormality, up to 92.6% among the women with HSIL cytology ($P = 0.0001$ for linear trend).

Until today, a large number of studies have been published, where performance indicators of Hybrid Capture II test have been analyzed in different settings (both screening and triage) [15,20–25,27–36,48,52,53,56]. An almost unanimous experience from these studies indicate that HCII test is more sensitive (above 95%) than PAP smear cytology (around 60–70%), but less specific (40–80%) than cytology (around 95–99%) in detecting high-grade CIN. Positive predictive value (PPV) of HCII test is usually low, but NPV approaches 100% in several of these studies. As repeatedly emphasized, however, these performance indicators are critically dependent on the prevalence of both HPV and CIN in the target population, and for this reason, quite different in a screening setting and in MAPS [21,22, 25,34,54]. The same also applies to performance indicators of other diagnostic tests, including PAP smear (LBC) cytology, VIA, and screening colposcopy [13–20], all depending on the prevalence of CIN lesions. The results in the present study are consonant with the published data, while showing that LBC has 99.5% specificity and 96% PPV in detecting CIN2–3, with sensitivity of 40% and NPV around 85% (Table 5). Also, the figures for colposcopy as a highly sensitive (96.5%) technique with high NPV (97.2%) are in alignment with our previous experience [48]. This implicates that the present MAPS Series is an appropriate cohort to compare the performance indicators of the AMPLICOR HPV test [37–39] with those previously reported for, e.g., HCII and PCR [15,20–25,27–36,48,52,53,56].

In the recent International Papillomavirus Congress (Mexico City 2004), several studies were presented, in which the Roche AMPLICOR® HPV test was validated in two different LBC media [37,38,44,47], and the performance of the test in different settings was compared with HCII, PCR, or cytology [39–43,45,46]. However, none of these studies have been published yet. Accordingly, Gibson et al. [39] compared AMPLICOR with HCII assay in a series of 987 randomly selected cervical samples collected in Thin-Prep and/or Sure-PATH media, and confirmed the discrepant cases with the Roche LINEAR ARRAY® HPV genotyping. In the study of van den Brule et al. [40], the AMPLICOR test was compared with the GP5+/GP6+ PCR assay in 98 cervical samples collected in PreservCyt® medium. The two tests gave very similar results, but the test sensitivity of the Roche AMPLICOR® HPV test appeared slightly higher [40].

In a series of 103 samples, the AMPLICOR test and HCII assay gave different results in 12 cases (11.6%),

showing a good correlation with the lesion grade and clinical outcome of the disease [41]. This is in agreement with our present data (Table 3), showing a linear relationship between the lesion grade and positive AMPLICOR test. The Roche AMPLICOR® HPV test and HCII assay gave practically identical results in borderline lesions, with 39/59 and 40/59 cases testing positive with the two tests, respectively [42]. In another series, 90/422 samples were positive and 269 were negative using both HCII and AMPLICOR tests, while 17 were positive only with the HCII assay and 46 tested positive only with the AMPLICOR test [43]. Ratnam compared HCII and AMPLICOR tests in a series of 208 cervical samples, and while both assays were 100% sensitive, the AMPLICOR test had markedly higher (95%) specificity as compared to that (74%) of the HCII test [45]. In a study, where 96 HCII positive samples and 100 HCII negative samples were analyzed by the AMPLICOR test using a modified type-specific PCR assay as the gold standard, sensitivity of AMPLICOR was 93.0% (95%CI 87.9–98.1) and that of HCII was 89.3% (83.0–95.5). Specificities for the two tests were 95.5% (91.2–99.9) and 92.7% (87.4–98.0), respectively [46].

In the present study, the Roche AMPLICOR HPV test had 95.2% (89.9–100.0) sensitivity, 42.4% (35.7–49.2) specificity, 33.7% (26.8–40.7) PPV, and 96.7% (93.0–100.0) NPV in detecting CIN2–3 lesions among women in the MAPS Series. These figures are practically identical with those of colposcopy (Table 5). These figures favorably compete with those reported for HCII assay as a triage tool [4,8,15,26–30]. Similar to HCII in most of the published studies, AMPLICOR HPV test cannot compete in specificity with LBC in detecting CIN. The analytical sensitivity of the Roche AMPLICOR HPV Test in detection of the 13 high-risk HPV types is higher (i.e., 480 copies/ml) than that of the HCII assay (around 5000 copies). Although the analytical sensitivity of the test is not invariably associated with similar difference in clinical sensitivity [23,34], the present data suggest that the sensitivity of the Roche AMPLICOR HPV test could be slightly higher than that of HCII, as determined from the published literature [4,8,15,26–30], and from the few reports where these two tests were directly compared [42,43,45,46]. This slight difference might be counterbalanced by the fact that HCII is fully automatic, whereas AMPLICOR is a more labor-intensive test in the laboratory. Overall, the test utility also depends on its purpose of use; in a screening setting, a more sensitive test (with high PPV) is advantageous, while in MAPS, the maximum specificity (and high NPV) should be the final goal. Like with HCII, the question about the appropriate cutoff for each purpose of test use [23,24] certainly merits additional discussion with the AMPLICOR test as well.

As the last step, we used logistic regression models to analyze the power of different variables as independent predictors of the outcome variables (SIL, CIN) in multivariate

models. The significant independent predictors of CIN2–3 in the MAPS Series were current PAP test at HSIL cutoff with OR 86.48 (95%CI 6.15–1215.28) ($P = 0.001$), positive AMPLICOR test with OR 32.38 (95%CI 3.69–284.13) ($P = 0.002$), positive colposcopy with OR 5.80 (95%CI 1.27–26.34) ($P = 0.023$), and the referral PAP at HSIL cutoff with OR 4.58 (95%CI 1.48–14.10) ($P = 0.008$). When the cutoff for positive colposcopy was increased from minor-change to borderline-change, the significance of this diagnostic tool was markedly increased to OR 10.64 (95%CI 4.67–24.26), and it negatively confounded (= increased) the predictive power of PAP test (HSIL) with OR 163.17 (95%CI 9.25–2877.38), but not that of 0AMPLICOR test. When colposcopy cutoff was settled at major-change, the predictive value of colposcopy in detecting CIN2–3 further increased to OR 14.56 (95%CI 5.61–37.78) ($P = 0.0001$), that of HSIL cytology to OR 125.53 (95%CI 8.00–1968.63) ($P = 0.001$), and that of AMPLICOR to OR 35.97 (95%CI 4.03–320.64) ($P = 0.001$). In both cases, the referral PAP lost its predictive power in the multivariate analysis.

As compared with the published data on HCII test, the Roche AMPLICOR® HPV test seems to be comparable to HCII assay in detecting high-grade CIN among women examined for abnormal PAP test. Test sensitivity in detecting both CIN1 and CIN2–3 is practically identical with the sensitivity of colposcopy (with minor abnormality cutoff), but like HCII, AMPLICOR assay cannot compete in specificity with cervical (LBC) cytology in this respect. Together with abnormal colposcopy and HSIL cytology, the AMPLICOR HPV test is a powerful independent predictor of high-grade CIN2–3, and as such highly suitable as a triage tool used in the management of abnormal PAP test. However, more data are clearly needed on the performance of AMPLICOR test in studies where it is directly compared with HCII as a triage tool, and particularly as a screening tool, based on biopsy-confirmed cervical pathology as the gold standard.

Acknowledgment

This study has been supported by a research grant from Roche Molecular Systems (France), kindly donating the AMPLICOR HPV tests at our disposal.

References

- [1] Ferlay J, Bray F, Pisani P, Parkin DM. GLOBOCAN 2000: cancer incidence, mortality and prevalence worldwide, version 1.0. IARC cancer base no. 5. Lyon: IARC Press; 2001.
- [2] Parkin DM, Pisani P, Ferlay J. Estimates of the worldwide incidence of 25 major cancers in 1990. *Int J Cancer* 1999;80:827–41.
- [3] Syrjänen K, Syrjänen S. Papillomavirus infections in human pathology. Chichester: J. Wiley and Sons; 2000. p. 1–615.
- [4] Aubin F, Pretet JL, Mougin C, editors. Papillomavirus humains. Biologie et pathologie tumorale. Paris: TEC and DOC; 2003. p. 1–759. Editions.
- [5] Miller AB, Robles SC. Workshop on screening for cancer of the uterine cervix in Central America. *Bull Pan Am Health Organ* 1996;30:397–408.
- [6] Miller AB, Nazeer S, Fonn S, Brandup-Lukanow A, Rehman R, Cronje H, et al. Report on consensus conference on cervical cancer screening and management. *Int J Cancer* 2000;86:440–7.
- [7] Franco E, Syrjänen K, de-Wolf C, Patnick J, Ferenczy A, McGoogan E, et al. New developments in cervical cancer screening and prevention. Geneva, Switzerland, June 17–19 1996, workshop. *Cancer Epidemiol, Biomarkers Prev* 1996;5:853–6.
- [8] Monsonego J, Bosch FX, Coursaget P, Cox JT, Franco E, Frazer I, et al. Cervical cancer control, priorities and new directions. *Int J Cancer* 2004;108:329–33.
- [9] Hakama M. Screening for cervical cancer: experience from the Nordic countries. In: Franco E, Monsonego J, editors. *New developments in cervical cancer screening and prevention*. Oxford: Blackwell Science; 1997. p. 190–9.
- [10] Ponten J, Adami H-O, Bergström R, Dillner J, Friberg L-G, Gustafsson L, et al. Strategies for global control of cervical cancer. *Int J Cancer* 1995;60:1–26.
- [11] Monsonego J. Global challenges of cervical cancer prevention. *Clin Exp Obstet Gynecol* 2001;28:5–13.
- [12] Patnick J, Monsonego J, de Wolf C, Verbeek A, Bonte J, Agnantis N, et al. ESGO consensus document on cervical cancer screening. European Society of Gynaecological Oncology. *Eur J Gynaecol Oncol* 2001;22:99–101.
- [13] Miller AB, Sankaranarayanan R, Bosch FX, Sepulveda C. Can screening for cervical cancer be improved, especially in developing countries? *Int J Cancer* 2003;107:337–40.
- [14] Sankaranarayanan R, Syamalakumary B, Wesley R, Amma NS, Parkin DM, Nair MK. Visual inspection with acetic acid in the early detection of cervical cancer and precursors. *Int J Cancer* 1999;80:161–3.
- [15] Costa S, Sideri M, Syrjänen K, Terzano P, De Nuzzo M, De Simone P, et al. Combined Pap smear, cervicography and HPV DNA testing in the detection of cervical intraepithelial neoplasia and cancer. *Acta Cytol* 2000;44:310–8.
- [16] Boselli F, De Martis S, Rivasi F, Toni A, Abbiati R, Chiossi G. The Italian experience of a Pap test and speculoscopy based screening programme. *J Med Screen* 2000;7:160–2.
- [17] Parham GP, Andrews NR, Lee ML. Comparison of immediate and deferred colposcopy in a cervical screening program. *Obstet Gynecol* 2000;95:340–4.
- [18] McGoogan E. Liquid-based cytology: the new screening test for cervical cancer control. *J Fam Plan Reprod Health Care* 2004;30:123–125.
- [19] Confortini M, Bonardi L, Bulgaresi P, Cariaggi MP, Cecchini S, Ciatto S, et al. A feasibility study of the use of the AutoPap screening system as a primary screening and location-guided rescreening device. *Cancer* 2003;99:129–34.
- [20] Cronje HS, Parham GP, Cooreman BF, de Beer A, Divall P, Bam RH. A comparison of four screening methods for cervical neoplasia in a developing country. *Am J Obstet Gynecol* 2003;188:395–400.
- [21] Cuzick J, Sasieni P, Davies P, Adams J, Normand C, Frater A, et al. A systematic review of the role of human papillomavirus testing within a cervical screening programme. *Health Technol Assess* 1999;3:1–204.
- [22] Franco EL. Chapter 13: primary screening of cervical cancer with human papillomavirus tests. *J Natl Cancer Inst Monogr* 2003;31:89–96.
- [23] Lörintz AT, Richart RM. Human papillomavirus DNA testing as an adjunct to cytology in cervical screening programs. *Arch Pathol Lab Med* 2003;127:959–68.
- [24] Bulkman NW, Rozendaal L, Snijders PJ, Voorhorst FJ, Boeke AJ, Zandwijken GR, et al. POBASCAM, a population-based randomized controlled trial for implementation of high-risk HPV testing in cervical screening: design, methods and baseline data of 44,102 women. *Int J Cancer* 2004;110:94–101.
- [25] Franco EL. Randomized controlled trials of HPV testing and Pap

- cytology: toward evidence-based cervical cancer prevention. *Int J Cancer* 2004;110:1–2.
- [26] Syrjänen KJ. Management of abnormal PAP smears (MAPS): implications of terminology used in cytopathology. *J Low Genit Tract Dis* 2000;4:217–23.
- [27] Arbyn M, Buntinx F, Van Ranst M, Paraskevaidis E, Martin-Hirsch P, Dillner J. Virologic versus cytologic triage of women with equivocal Pap smears: a meta-analysis of the accuracy to detect high-grade intraepithelial neoplasia. *J Natl Cancer Inst* 2004;96:280–93.
- [28] Carozzi FM, Confortini M, Cecchini S, Bisanzio S, Cariaggi MP, Pontenani G, et al. Triage with human papillomavirus testing of women with cytologic abnormalities prompting referral for colposcopy assessment. *Cancer* 2005;105:2–7.
- [29] Massad LS, Schneider MF, Watts DH, Strickler HD, Melnick S, Palefsky J, et al. HPV testing for triage of HIV-infected women with papanicolaou smears read as atypical squamous cells of uncertain significance. *J Womens Health (Larchmt)* 2004;13:147–53.
- [30] Solomon D, Schiffman M. Have we resolved how to triage equivocal cervical cytology? *J Natl Cancer Inst* 2004;96:250–1.
- [31] Castle PE, Wheeler CM, Solomon D, Schiffman M, Peyton CL. Interlaboratory reliability of Hybrid Capture 2. *Am J Clin Pathol* 2004;122:238–45.
- [32] Federschneider JM, Yuan L, Brodsky J, Breslin G, Betensky RA, Crum CP. The borderline or weakly positive Hybrid Capture II HPV test: a statistical and comparative (PCR) analysis. *Am J Obstet Gynecol* 2004;191:757–61.
- [33] Giovannelli L, Lama A, Capra G, Giordano V, Arico P, Ammatuna P. Detection of human papillomavirus DNA in cervical samples: analysis of the new PGMY-PCR compared to the hybrid capture II and MY-PCR assays and a two-step nested PCR assay. *J Clin Microbiol* 2004;42:3861–4.
- [34] Kulmala SM, Syrjänen S, Shabalova I, Petrovichev N, Kozachenko V, Podistov J, et al. Human papillomavirus testing with the hybrid capture 2 assay and PCR as screening tools. *J Clin Microbiol* 2004;42:2470–5.
- [35] Nonogaki S, Wakamatsu A, Longatto Filho A, Pereira SM, Utagawa ML, Ferreira Alves VA, et al. Hybrid capture II and polymerase chain reaction for identifying HPV infections in samples collected in a new collection medium: a comparison. *Acta Cytol* 2004;48:514–20.
- [36] Pretet JL, Dalstein V, Monnier-Benoit S, Delpeut S, Mougín C. High risk HPV load estimated by Hybrid Capture II correlates with HPV16 load measured by real-time PCR in cervical smears of HPV16-infected women. *J Clin Virol* 2004;31:140–7.
- [37] Cubie HA, Cuschieri KS, Moore C. Stability of Human papillomavirus (HPV) DNA in PreservCyt® at 4°C and room temperature, as detected by the Roche AMPLICOR® HPV test. 21st International Papillomavirus Conference, Mexico City, February 21–26. Abstract.
- [38] Kosarikov D, Ahmad A, Current R, Ding K, Igdari S, Joshi S, et al. Stability of liquid PAP specimens for the detection of high-risk Human papillomavirus (HPV) types with the Roche AMPLICOR® HPV test. 21st International Papillomavirus Conference, Mexico City, February 21–26. Abstract.
- [39] Gibson J, Vinson D, Flynn D, Garcia R, Iaquín D, Kohler M, et al. The Roche AMPLICOR® HPV test as a primary screen for HPV in cervical specimens collected in Thin-Prep® and Sure-Path™ Liquid cytology media. 21st International Papillomavirus Conference, Mexico City, February 21–26. Abstract.
- [40] van den Brule AJ, Moerland E, Wiering P, van Toren J, Klinkhamer PJM. Comparison of the Roche AMPLICOR® Human papillomavirus (HPV) test with the GP5+/GP6+PCR HPV assay in cervical samples with known cytology and histology. 21st International Papillomavirus Conference, Mexico City, February 21–26. Abstract.
- [41] Lindauer A, Böhmer G, Menzel K, Rinnau F, Petry KU. AMPLICOR® (and line strip) HPV-testing in patients with CIN. 21st International Papillomavirus Conference, Mexico City, February 21–26. Abstract.
- [42] Cubie HA, Kate S, Cuschieri F, Zmijewski M, Moore C. Evaluation of the sensitivity and specificity of the Roche AMPLICOR® Human papillomavirus (HPV) test, the Roche Linear Array® HPV test and the Digene Hybrid Capture 2 assay for the detection of HPV in archived cervical samples with borderline cytology. 21st International Papillomavirus Conference, Mexico City, February 21–26. Abstract.
- [43] Sargent A, Bailey A, Wheeler P, Kitchener H, Corbitt G. A comparison of the Digene Hybrid Capture 2 assay and the Roche AMPLICOR® Human papillomavirus (HPV) test for the detection of high-risk HPV genotypes in DNA extracts from liquid-based cytology samples collected from women whose cytology was graded borderline. 21st International Papillomavirus Conference, Mexico City, February 21–26. Abstract.
- [44] Beyser K, Schön U, Lindauer A. Reliability of the Roche AMPLICOR® Human papillomavirus (HPV) test. 21st International Papillomavirus Conference, Mexico City, February 21–26. Abstract.
- [45] Ratnam S. Performance of Roche AMPLICOR® Human papillomavirus (HPV) test in comparison with the Digene Hybrid Capture 2 assay for the detection of high-risk oncogenic HPV in cervical specimens. 21st International Papillomavirus Conference, Mexico City, February 21–26. Abstract.
- [46] Young S, Mills R, Taylor K. An initial performance evaluation of the AMPLICOR® Human papillomavirus (HPV) test, the linear array HPV test and the Digene Hybrid Capture 2 high-risk HPV DNA tests for detection of high-risk HPV. 21st International Papillomavirus Conference, Mexico City, February 21–26. Abstract.
- [47] Sun R, Current R, Ding K, Joshi K, Kotadia R, Kosarikov D, et al. Evaluation of the Roche AMPLICOR® Human papillomavirus (HPV) test. 21st International Papillomavirus Conference, Mexico City, February 21–26. Abstract.
- [48] Monsonego J, Javier Pintos J, Semaille C, Beumont M, Dachez R, Zerat L, et al. HPV testing improves the accuracy of colposcopy in detection of cervical intraepithelial neoplasia. *Int J Gynecol Oncol* [in press].
- [49] Walker P, Dexeus S, De Palo G, Barrasso R, Campion M, Girardi F, et al. Nomenclature committee of the international federation for cervical pathology and colposcopy. International terminology of colposcopy: an updated report from the International Federation for Cervical Pathology and Colposcopy. *Obstet Gynecol* 2003;101:175–7.
- [50] Fleiss JL, Cohen J. The equivalence of weighted kappa and the intraclass correlation coefficient as measures of reliability. *Educ Psychol Meas* 1973;33:613–9.
- [51] Norusis JM. Measures of reliabilities in scale problems, Chapter 17. SPSS base 10.0 applications guide. Chicago: SPSS Inc; 1999. p. 359–68.
- [52] Clavel C, Masure M, Levert M, Putaud I, Mangeonjean C, Lorenzato M, et al. Human papillomavirus detection by the hybrid capture II assay: a reliable test to select women with normal cervical smears at risk for developing cervical lesions. *Diagn Mol Pathol* 2000;9:145–50.
- [53] Clavel C, Masure M, Bory JP, Putaud I, Mangeonjean C, Lorenzato M, et al. Hybrid Capture II-based human papillomavirus detection, a sensitive test to detect in routine high-grade cervical lesions: a preliminary study on 1518 women. *Br J Cancer* 1999;80:1306–11.
- [54] Syrjänen S, Shabalova IP, Petrovichev N, Kozachenko VP, Zakharova T, Pajanidi A, et al. Sexual habits and Human papillomavirus (HPV) infections among women in three new independent states of the former Soviet Union. *Sex Transm Dis* 2003;30:680–4.
- [55] Monsonego J, Autillo-Touati A, Bergeron C, Dachez R, Liaras J, Saurel J, et al. Liquid-based cytology for primary cervical cancer screening: a multi-centre study. *Br J Cancer* 2001;84:360–6.
- [56] Human papillomavirus testing for triage of women with cytologic evidence of low-grade squamous intraepithelial lesions: baseline data from a randomized trial. The Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesions Triage Study (ALTS) Group. *J Natl Cancer Inst* 2000;92:397–402.