

Self-sampling for human papillomavirus (HPV) testing as cervical cancer screening option. Experience from the LAMS Study

**A. Longatto-Filho^{1,2}, M.Sc., Ph.D., PMAC; C. Roteli-Martins³, M.D., Ph.D.; L. Hammes⁴, M.D., Ph.D.;
D. Etlinger¹, B.Sc.; S.M. Miranda Pereira¹, B.Sc.; M. Eržen⁵, M.D., Ph.D., MIAC;
M. Branca⁶, M.D., Ph.D., MIAC; P. Naud⁴, M.D., Ph.D.; S.F.M. Derchain⁷, M.D., Ph.D.;
L.O. Sarian⁷, M.D.; J. Matos⁴, M.D.; R. Gontijo⁷, M.D.; T. Lima³, M.D.; M.Y.S. Maeda¹, M.Sc.;
S. Tatti⁸, M.D., Ph.D.; S. Syrjänen⁹, D.D.S., Ph.D.; K. Syrjänen¹⁰, M.D., Ph.D., FIAC**

¹Instituto Adolfo Lutz, São Paulo (Brazil)

²Life and Health Sciences Research Institute, School of Health Sciences, University of Minho, Braga (Portugal)

³Hospital Leonor Mendes de Barros, São Paulo (Brazil)

⁴Hospital de Clinicas de Porto Alegre (Brazil); ⁵SIZE Diagnostic Center, Ljubljana (Slovenia)

⁶Istituto Superiore di Sanità, Rome (Italy); ⁷Universidade Estadual de Campinas (Brazil)

⁸First Chair Gynecology Hospital de Clinicas, BA (Argentina); ⁹Department of Oral Pathology, University of Turku (Finland)

¹⁰Department of Oncology & Radiotherapy, University of Turku (Finland)

Summary

Purpose: To compare Hybrid Capture II (HC2) in detecting high-risk (HR) HPV in patient-collected vaginal samples with those obtained using gynaecologist collected samples. **Methods:** Patients were submitted to Pap smears, visual inspection with acetic acid (VIA) and HC2 for hr-HPV. **Results:** A total of 1,081 HC2 tests for HR-HPV were performed: 770 (71.2%) samples were collected by a physician and 311 (28.8%) were self-collected by the patients. In detecting any cervical lesion, the sensitivity of HC2 collected by a physician was higher (92.86%) than that (37.5%) in the self-sampling group. Negative predictive value (NPV) was high for both, 99.69% and 93.75%, respectively. Using the CIN2 cutoff, performance of HC2 was significantly improved: 92.9% and 62.5%, respectively. HC2 specificity for any cervical lesion and for CIN2 or higher were close to 90% in both groups. **Conclusions:** Self-sampled HPV testing is a powerful option to increase the detection of cervical lesions in women segregated from prevention programs.

Key words: Hybrid capture; HPV; Cervical cancer; Liquid based cytology; Self-sampling.

Introduction

Human papillomavirus (HPV) is the most widespread sexually transmitted disease (STD), with an estimated global prevalence of 10.4% among women with normal cytology, although substantial differences are encountered in different regions [1]. Relatively few high-risk HPV types (HR-HPV), most notably HPV16 and HPV18, are associated with more than 99% of all cervical carcinomas [2].

Effective prevention of cervical cancer with organized cytology-based screening programs necessitates well-trained professionals with different skills. Until now, such programs have only been implemented in some highly developed countries [3], and on the global scale, the vast majority of women diagnosed as having cervical cancer have never participated in organized cytological screening [4]. Since the demonstration of HR-HPV types as the necessary cause of cervical cancer [5], and recognition that cervical cytology suffers from low sensitivity, the use of HPV testing by Hybrid Capture II (HC2) was approved

by the United States Food and Drug Administration (FDA) in 2003 to be used concomitantly with cytology or alone [6]. In view of these facts, HR-HPV testing has been a part of new strategies for the screening of HPV induced-lesions in the US [7].

A variety of self-sampling devices have been introduced for collection of vaginal samples for HC2 testing. These systems have been tested in several studies, and shown to be a potentially viable screening option for women outside the regular programs of screening [8-11]. Indeed, the sensitivity of such self-collected vaginal samples for HPV testing has varied from 66.1% to 90% [3, 8-11]. Based on this experience, self-sampling for HPV testing seems a promising first-line option in cervical cancer screening, particularly in settings where cervical cytology is not readily available or insufficient in quantity to ensure wide enough coverage of the whole female population. In this setting, only women testing positive for hr-HPV should be referred for additional examinations [3]. Additionally, a high level of concordance between self-collected samples and physician sampling have been experienced. Restricting the results in HR-HPV, the concordance remains high but in contrast, low-risk HPV is more frequently identified in self-collected samples [11].

Revised manuscript accepted for publication October 1, 2007

Interestingly, women generally found the self-sampling option more suitable than the test performed by clinicians, but they were not confident that the test had been done properly [12]. Important demographic differences were also reported, e.g., married women having more positive attitudes towards self-sampling than single women, and Asian women having more negative attitudes than women in other ethnic groups [12]. Adolescents and young adult women seemed to prefer clinician to self-testing, largely because of concerns about self-collection accuracy [13]. These observations are essential issues to be considered by the authorities who want to plan self-sampling HPV testing as an alternative tool for primary screening, because a remarkably high prevalence of hr-HPV (three to six times higher than the expected prevalence in women of comparable age) can occur; apparently, these results closely depend on to the skill of the population analysed [13, 14].

In spite of encouraging data, there are several divergent results regarding the agreement between clinician- and self-collected vaginal samples for HPV, and the sensitivity value of HPV clinician testing and self-testing to detect cervical lesion [14]. Most of the disagreements discussed above may be largely related to differences in recruitment and data collection procedures, study populations, analytical methods and outcome measures [14].

In our ongoing multi-center study in Latin America, a cohort of over 12,000 women have been examined using eight different diagnostic tests as potential screening tools in low-resource settings. The main objective of this study was to compare the results of HC2 assay (for HR-HPV) in two types of samples: i) patient-collected vaginal samples, and ii) samples collected by gynecologists.

Materials and Methods

The enrolled cohort is part of the Latin American Screening (LAMS) study, a prospective multicenter cohort study that tested optional cervical cancer screening methods and assessed the natural history of HPV infections and CIN in four clinical centers in Brazil (Leonor Mendes de Barros Hospital, HLMB; Hospital de Clínicas de Porto Alegre and State University of Campinas) and Argentina (First Chair, Gynecology Hospital of Clinics). The study design and the baseline data of the LAMS study have been detailed recently [15].

The present analysis comprises the HLMB cohort only. In this cohort, patients were screened for cervical cancer with Pap smears, visual inspection with acetic acid (VIA) and HC2 for high risk HPV (HR-HPV).

HC2 was collected by a physician or by a self-sampling method and inclusion of the patient in either group was randomly performed. The self-collected sample by the patient was performed after preliminary oriented-instruction by a well-trained nurse. General characteristics of the patients were reported.

Women testing positive for any of the tests were referred for colposcopy, and cervical biopsies were performed if necessary.

All patients gave their written consent to participate in the study, which was approved by the local Ethics Committee.

Histological specimens

All cases referred for colposcopy and biopsies were taken according to clinical evaluation. The cases were primarily classified according to WHO's 1994 classification [16] and, afterwards revised according WHO's 2003 classification [17].

Hybrid Capture Assay

The HC2 protocol was performed according to the instructions of the manufacturer (Digene Co., Gaithersburg, MD, USA). In estimation of the viral load, samples with relative light units (RLU) > 20 were considered to harbor a high viral load and, those with 5-19.9 were intermediate, and those with 1-4.99 were low [18]. Only HR-HPV was tested (carcinogenic types included: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) [19].

Statistical methods

In statistical analyses, two different statistical softwares were used: SPSS for Windows (Version 11.5) and STATA/SE 8.2. The performance indicators (sensitivity, SE, specificity, SP, negative predictive value NPV, and positive predictive value, PPV) for conventional Pap tests and liquid based cytology (LBC) were calculated from the 2 x 2 contingency tables, using colposcopic biopsies as the gold standard. The chi-square test was used to analyze correlations between categorical data, with Pearson's correlation and Fisher's exact test, and calculating OR and their 95% CI where appropriate. In all statistical analyses $p < 0.05$ was regarded as significant.

Results

A total of 1,081 tests of HC2 for hr-HPV were performed: 770 (71.2%) samples were collected by a physician and 311 (28.8%) were self-collected by the patients.

Table 1 shows the principal characteristics of the patients regarding age, years of education, age at first intercourse, number of pregnancies, number of deliveries or caesarean sections, number of abortions (prenatal births), number of partners since first intercourse and during the previous 12 months, and the number of Pap tests during the lifetime. Interestingly, the values of both groups were quite similar. Mean and median age were around 37 years. The other parameters revealed that the women in our case series have comparable cultural attitudes.

Table 2 depicts the race distribution which revealed a significant difference among women in both groups (sampled by a physician or self-collected samples). High-risk HPV infection in white women was more prevalent in comparison to the results observed in black and mixed ($p = 0.0001$). No other variable was significantly different in either group, including contraception methods, which demonstrates the homogeneity of the women's history regarding contraceptive usage, and previous history of sexually transmitted disease.

Table 3 exhibits the results of hr-HPV Pap smear examination, HC2 tests and VIA correlated to the method of sample collection. All parameters were more significantly positive in the self-sampling group than in material sampled by a physician. The differences were particularly interesting in cases with any cytological abnormality ($p = 0.008$) and in positive VIA ($p = 0.0001$).

Table 1. — Quantitative history variables of the patients tested for hr-HPV by the two sampling methods for HC2 assay.

Characteristics	Method of HC2 sampling		P*
	HC2 sampling by physician Mean \pm Std. deviation	HC2 self-sampling Mean \pm Std. deviation	
Age	37.55 \pm 9.73	36.97 \pm 9.97	0.384
Years of education	7.1 \pm 3.5	7.5 \pm 3.5	0.11
Age at first sexual intercourse	18.5 \pm 3.8	18.9 \pm 4.5	0.697
No. of pregnancies	2.8 \pm 2.2	2.5 \pm 1.9	0.017
No. of deliveries	1.7 \pm 1.9	1.4 \pm 1.6	0.058
No. of cesarean sections	0.6 \pm 1.0	0.7 \pm 1.0	0.63
No. of abortions/ prenatal births	0.5 \pm 0.9	0.4 \pm 0.8	0.029
No. of partners since the first intercourse	2.5 \pm 3.3	2.4 \pm 2.1	0.058
No. of partners during the past 12 months	1.0 \pm 0.4	1.0 \pm 0.7	0.959
No. of life-time Pap smears	6.5 \pm 4.8	6.6 \pm 5.2	0.707

* Mann-Whitney U-test; HC2, Hybrid capture II; HR-HPV, high-risk human papillomavirus.

Table 2. — Race, contraception, STD and smoking history of patients tested for HR-HPV by the two sampling methods for HC2 assay.

Characteristics	Method of HC sampling				P
		HC2 sampling by physician N (%)	HC2 self-sampling N (%)		
Race	White	511 (66.5%)	194 (62.4%)		
	Black	71 (9.2%)	53 (17%)		
	Mixed	164 (21.4%)	50 (16.1%)		
	Other	22 (66.5%)	14 (4.5%)		0.001*
Contraception	none	216 (28.1%)	90 (28.9%)		
	hormonal	193 (25.1%)	76 (24.4%)		
	condom	109 (14.2%)	55 (17.7%)		
	IUD	67 (8.7%)	26 (8.4%)		
	tubal sterilization	142 (18.5%)	50 (17.8%)		
History of STD	other	42 (5.5%)	14 (5.2%)		0.696*
	Patient	55 (7.2%)	18 (5.8%)		0.5**
	Partners	68 (8.8%)	22 (7.1%)		0.406**
Previous pap smear		718 (93.4%)	297 (95.5%)		0.233**
Smoking (current or past)		279 (36.3%)	108 (34.7%)		0.680**

* Pearson's chi-square; ** Pearson's chi-square with continuity correction; HC2, Hybrid capture II; HR-HPV, high-risk human papillomavirus; IUD, intrauterine contraceptive device; STD, sexually transmitted disease.

Table 3. — Results of Pap smear, HC2 for HR-HPV and VIA in the two groups of sampling.

Exam		Method of HC sampling		P*
		HC2 sampling by physician N/total (%)	HC2 self-sampling N (%)	
Pap	ASCUS or higher	72/770 (9.4%)	47/311 (15.1%)	0.008
	HSIL or higher	10/770 (1.3%)	12/311 (3.9%)	0.014
HC2	Positive	108/770 (14%)	63/311 (20.3%)	0.014
VIA	Positive	89/770 (11.6%)	64/311 (20.6%)	0.000

* Pearson chi-square; HC2, Hybrid capture II; HR-HPV, high-risk human papillomavirus; VIA, Visual inspection with acetic acid.

Table 4 demonstrates the correlation of HC2 positive tests related to the final diagnoses in the two groups of sampling. It is important to observe that among the samples collected by a physician only one case of CIN2 or higher was negative; in contrast, three cases were missed in self-collected samples. Conversely, healthy cervix or CIN1 cases were consistently negative by HC2 tests in both groups (97.4% and 95.6% in physician-collected sampling and self-sampling, respectively).

Finally, Table 5 shows the biopsy-proven performance of HC2 testing for HR-HPV in both sampling methods. Considering any cervical lesion, the sensitivity of HC2 collected by the physician was higher than that observed in the self-sampling group (92.86% and 37.5%, respectively). However, the NPV was importantly high for both (99.69% and 93.75%, respectively). Using a more restrictive cutoff, CIN2 or higher, the performance was significantly enhanced: 92.86% and 62.5%, respectively. It is important to emphasize that for both sampling methods the 95% CI was very small, which reinforces the robustness of the HC2 method in both sampling situations. Similarly, and remarkably, the HC2 specificity values for any lesion higher than ASCUS and for CIN2 or higher were very high (close to 90%) and also presented a small 95% CI.

Discussion

HPVs infect epithelial cells and cause a variety of lesions including warts to cervical neoplasia and cancer. High-risk HPV DNA is found in almost all cervical cancers (> 99.7%), with HPV16 being the most prevalent type in both low-grade disease and cervical neoplasia [20]. Identifying HR-HPV in women with cervical cancer is critical to understanding the pathogenesis of cervical cancer [21]. Presently, the identification of HR-HPV has been determinedly advocated by epidemiologists who are clearly identifying the correlation between HR-HPV infection and cervical high-grade lesions [1, 7, 11, 22, 23].

The complexity of cytologic-based screening and the necessity of resources, infrastructure, professional expertise, together with the need for repeated and well-controlled screenings at regular intervals, make cervical cytologic screening very difficult to be efficiently implemented in poor countries [22]. Additionally, the accuracy and reproducibility of the Pap test is far from acceptable as the primary screening option in low resource settings [24]. Recognition of HR-HPV DNA is also important to improve the identification of cervical lesions alone or associated with cytological examination [25, 26]. Recently, we studied HC2 as an optional tool for primary screening, and the results clearly demonstrated this tendency due to the superior correlation of positive hr-HPV testing with biopsy-proven high-grade lesions when compared with cytology, conventional or liquid-based preparations [27]. Importantly, the HC2 option seems to be more cost-effective than cytology and its use is encouraged for low resource countries [7, 28-31], and it is more accurate for women aged 30 years or more [7, 29]. HC2

Table 4. — HC2 results related to the final diagnosis in the two groups of sampling.

Final diagnosis	HC2 sampling by physician		Method of HC sampling		HC2 self-sampling	
	Positive	Negative	Positive	Negative	Positive	Negative
CIN2 or higher	13 (12%)	1 (0.2%)	5 (7.9%)	3 (1.2%)	36 (57.2%)	237 (95.6%)
Healthy cervix or CIN1	73 (67.6%)	645 (97.4%)	22 (34.9%)	8 (3.2%)	63 (100%)	248 (100%)
Screening not completed	22 (20.4%)	16 (2.4%)	5 (7.9%)	3 (1.2%)	63 (100%)	248 (100%)
Total	108 (100%)	662 (100%)	63 (100%)	248 (100%)	63 (100%)	248 (100%)
P *	< 0.001		< 0.001		< 0.001	

* Pearson chi-square; HC2, Hybrid capture II; HR-HPV, high-risk human papillomavirus; CIN, cervical intraepithelial neoplasia.

Table 5. — Performance indicators of HC2 testing in detecting cervical lesions in the two groups *.

Histology cutoff	Statistical index **	Method of HC2 sampling		HC2 self-sampling	
		HC sampling by physician Value	Value (IC95%)	Value	Value (IC95%)
Any lesion***	Sensitivity	92.86%	75.0-98.8	37.50%	19.5-59.2
	Specificity	91.48%	89.1-93.4	87.55%	82.7-91.2
	Positive predictive value	30.23%	21.0-41.2	21.95%	11.1-38.0
	Negative predictive value	99.69%	98.8-99.9	93.75%	89.7-96.3
CIN2 or higher	Sensitivity	92.86%	64.2-99.6	62.50%	25.9-89.8
	Specificity	89.83%	87.3-91.9	86.81%	82.1-90.5
	Positive predictive value	15.12%	8.6-24.8	12.20%	4.6-27.0
	Negative predictive value	99.85%	99.0-100.0	98.75%	96.1-99.7

* Cases that did not complete screening were not included in this analysis; ** For all comparisons; Pearson's chi-square < 0.001; *** Any lesion = HPV infection, cervical intraepithelial neoplasia (CIN) or carcinoma.

hr-HPV also has an important predictive impact for both negative and positive results. Oncogenic HPV infections comprise a significant risk factor for incident cervical abnormalities [32-35]. Remarkably, among older women where HPV may be added to general screening, the estimated absolute risk of high grade lesions in HC2-positive women is believed to be superior than 20% within ten years which indicates that even a single positive HPV test in cytologically negative women is substantially predictive of high-grade CIN; this fact supports the use of HC2 testing to stratify women into different risk categories [36].

In this context, self-sampling screening could be an important option to select cervical lesions in women out of the regular health system programs in poor regions of developing countries. There are several data that robustly demonstrated this potential [4, 8-13], including in Brazil [3]. Complementary, the performance of HC2 for HR-HPV in self-collected material and those collected by a physician was slightly different. In the physician material the sensitivity was significantly superior to that found in self-collected samples but the NPV in both groups was quite similar. The values observed are concordant to those observed in recent meta-analyses [11]. A high level of concordance of 0.87 (95% CI, 0.82 to 0.91) was observed between self- and physician-sampling for detection of HPV DNA (Kappa 0.66, 95% CI, 0.56 to 0.76). Self-sampling was demonstrated as sensitive as physician-obtained sampling to detect HR-HPV or HPV DNA.

Our results endorse the findings previously reported and open a new route of cervical cancer prevention to be considered for public health authorities. Self-sampling may be also a useful option for studies on HPV transmis-

sion and vaccine trials [11]. Considering circumstances where there are important difficulties in assessing women who are out of the regular prevention cervical cancer programs strongly favors the use of the self-sampling method to evaluate hr-HPV among these women [10].

In spite of several efforts, cervical cancer is still the leading cause of morbidity and mortality in Brazil [37]. The last three decades has not shown any significant results in decreasing mortality in Brazil. Recent analyses published by INCA (Brazilian National Institute Against Cancer) estimated for 2006 19,260 new cases, or 20 cases for 100,000 women (www.inca.gov.br). Contrary to these terrible facts there is no discussion that opportunistic or organized screening based only on cytological screening is insufficient to preclude thousands of preventable deaths. The uses of human papillomavirus DNA tests, alone or combined with cytology, are now recommended by INCA in Brazil [37].

Noteworthy conclusions can be assessed with our results. Self-sampling is a reliable tool for women to collect material for HC2 analysis. Even with a performance slightly inferior to those obtained by a physician, the ability to collect optimal samples by women was clearly ratified in that HC2 can be performed elsewhere with confident performance. These observations are similar to those reported by Holanda and co-workers in Brazil [3].

Importantly, HC2 for HR-HPV showed a consistent and superior performance when compared with other screening options, including cytology, as we have already observed previously [27]. Additionally, HC2 showed high sensitivities to detect CIN2 or higher lesions (92.86%) which support the high clinical sensitivity of HC2 tests to

identify high-grade lesions. Moreover, the NPV was almost 100% for CIN2 or higher (99.85%) which evidently demonstrates that HR-HPV testing negative with HC2 in self-sampling material is a safe and reliable resource for population screening. Importantly, the specificity for high grade lesions was superior by almost 90% implicating an additional gain for the self-sampling option. Our results found comparable values in the literature which strongly support the reproducibility of HC2 for HR-HPV collected by the self-sampling method [38, 39].

Conclusion

Self-sampling screening in remote areas of developing countries should be seriously considered as a powerful tool to reduce the prevalence of cervical cancer and its high-grade precursors, and to cooperate with the efforts to decrease mortality [3]. Obviously, these assumptions must be further measured in screening programs to test the efficiency in a large population.

Acknowledgments

This study is a part of the ongoing LAMS (Latin America Screening) study, entitled: IMPROVING HEALTH SYSTEMS TOWARDS EQUALITY-BASED CONTROL OF CERVICAL CANCER IN LATIN AMERICA. Comparing Pap smear cytology, aided visual inspection, cervicography and human papillomavirus (HPV) testing as optional screening tools in Brazil and Argentina, and supported by the INCO-DEV Programme of the European Commission (Project #ICA4-CT-2001-10013). The kind cooperation of all professionals of all institutions involved in this project is gratefully acknowledged. The authors also express their special thanks to DIGENE Corporation for providing the Hybrid Capture Kits at our disposal.

References

- [1] Burchell A.N., Winer R.L., de Sanjose S., Franco E.L.: "Chapter 6: Epidemiology and transmission dynamics of genital HPV infection". *Vaccine*, 2006, 24 (suppl. 3), S52.
- [2] Villa L.L.: "CHAPTER 1 Biology of genital human papillomaviruses". *Int. J. Gynaecol. Obstet.*, 2006, 94 (suppl. 1), S3.
- [3] Holanda F. Jr., Castelo A., Veras T.M., de Almeida F.M., Lins M.Z., Dore G.B.: "Int. J. Primary screening for cervical cancer through self sampling". *Gynaecol. Obstet.*, 2006, 95, 179.
- [4] Stenvall H., Wikstrom I., Backlund I., Wilander E.: "Accuracy of HPV testing of vaginal smear obtained with a novel self-sampling device". *Acta Obstet. Gynecol. Scand.*, 2007, 86, 16.
- [5] Moscicki A.B., Schiffman M., Kjaer S., Villa L.L.: "Chapter 5: Updating the natural history of HPV and anogenital cancer. *Vaccine*", 2006, 24 (suppl. 3), S42.
- [6] FDA news. FDA approves expanded use of HPV Test. March 31, 2003. www.fda.gov/bbs/topics/news/2003/new00890.html
- [7] Saslow D., Castle P.E., Cox J.T., Davey D.D., Einstein M.H., Ferris D.G. et al.: "American Cancer Society Guideline for Human Papillomavirus (HPV) Vaccine Use to Prevent Cervical Cancer and Its Precursors". *C.A. Cancer J. Clin.*, 2007, 57, 7.
- [8] Hillemanns P., Kimmig R., Hüttemann U., Dannecker C., Thaler C.J.: "Screening for cervical neoplasia by self-assessment for human papillomavirus DNA". *Lancet*, 1999, 354, 1970.
- [9] Wright T.C., Denny L., Kuhn L., Pollack A., Lorincz A.: "HPV DNA testing of self-collected vaginal samples compared with cytologic screening to detect cervical cancer". *JAMA*, 2000, 283, 81.
- [10] Szarewski A., Cadman L., Mallett S., Austin J., Londesborough P., Waller J. et al.: "Human papillomavirus testing by self-sampling: assessment of accuracy in an unsupervised clinical setting". *J. Med. Screen.*, 2007, 14, 34.
- [11] Petignat P., Faltin D.L., Bruchim I., Tramer M.R., Franco E.L., Coutlee F.: "Are self-collected samples comparable to physician-collected cervical specimens for human papillomavirus DNA testing? A systematic review and meta-analysis". *Gynecol. Oncol.*, 2007, 105, 530.
- [12] Waller J., McCaffery K., Forrest S., Szarewski A., Cadman L., Austin J., Wardle J.: "Acceptability of unsupervised HPV self-sampling using written instructions". *J. Med. Screen.*, 2006, 13, 208.
- [13] Dannecker C., Siebert U., Thaler C.J., Kiermeir D., Hepp H., Hillemanns P.: "Primary cervical cancer screening by self-sampling of human papillomavirus DNA in internal medicine outpatient clinics". *Ann. Oncol.*, 2004, 15, 863.
- [14] Kahn J.A.: "Self-testing for human papillomavirus using a vaginal swab: placing prevention of cervical cancer in the patient's hands". *Ann. Oncol.*, 2004, 15, 847.
- [15] Syrjänen K., Naud P., Derchain S., Roteli-Martins C., Longatto-Filho A., Tatti S. et al.: "Comparing Pap smear cytology, aided visual inspection, screening colposcopy, cervicography and HPV testing as optional screening tools in Latin America. Study design and baseline data of the LAMS study". *Anticancer Res.*, 2005, 25, 3469.
- [16] Scully R.E., Bonfiglio T.A., Silverberg S.G., Wilkinson E.J.: "World Health Organization International Classification of Tumours. Histological Typing of Female Genital Tract Tumours". 2nd edition, Berlin, Springer-Verlag, 1994.
- [17] Tavassoli F.A., Deville P.: "Tumours of the Breast and Female Genital Organs. World Health Organization Classification of Tumours". Lyon, IARC Press, 2003.
- [18] Cox J.T., Lorincz A.T., Schiffman M.H., Sherman M.E., Cullen A., Kurman R.J.: "Human papillomavirus testing by hybrid capture appears to be useful in triaging women with cytologic diagnosis of atypical squamous cells of undetermined significance". *Am. J. Obstet. Gynecol.*, 1995, 172, 946.
- [19] Howard M., Sellors J., Kaczorowski J., Lorincz A.: "Optimal cutoff of the hybrid capture II human papillomavirus test for self-collected vaginal, vulvar, and urine specimens in a colposcopy referral population". *J. Lower Genital Tract. Dis.*, 2004, 8, 33.
- [20] Doorbar J.: "Molecular biology of human papillomavirus infection and cervical cancer". *Clin. Sci (Lond)*, 2006, 110, 525.
- [21] Longworth M.S., Laimins L.A.: "Pathogenesis of human papillomaviruses in differentiating epithelia". *Microbiol. Mol. Biol. Rev.*, 2004, 68, 362.
- [22] Goldie S.: "CHAPTER 10 A public health approach to cervical cancer control: Considerations of screening and vaccination strategies". *Int. J. Gynaecol. Obstet.*, 2006, 94 (suppl. 1), S95.
- [23] Cuzick J., Clavel C., Petry K.U., Meijer C.J., Hoyer H., Ratnam S. et al.: "Overview of the European and North American studies on HPV testing in primary cervical cancer screening". *Int. J. Cancer*, 2006, 1095.
- [24] Nanda K., McCroy D.C., Myers E.R., Bastian L.A., Hasselblad V., Hickey J.D., Matchar D.B.: "Accuracy of the Papanicolaou test in screening for and follow up of cervical cytologic abnormalities: a systematic review". *Ann. Intern. Med.*, 2000, 132, 810.
- [25] Witt A., Hudelist G., Gregor H., Kucera E., Walchetseder C.: "Czerwenka. The detection of HPV DNA improves the recognition of cervical intraepithelial lesions". *Arch. Gynecol. Obstet.*, 2003, 268, 29.
- [26] Arbyn M., Buntinx F., Ranst M.V., 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". *JNCI*, 2004, 96, 280.
- [27] Longatto-Filho A., Erzen M., Branca M., Roteli-Martins C., Naud P., Derchain S.F., Hammes L., Sarian L.O., Braganca J.F., Matos J., Gontijo R., Lima T., Maeda M.Y., Tatti S., Syrjänen S., Dore G., Lorincz A., Syrjänen K.: "Human papillomavirus testing as an optional screening tool in low-resource settings of Latin America: experience from the Latin American Screening study". *Int. J. Gynecol. Cancer*, 2006, 16, 955.

- [28] Goldie S.J., Kuhn L., Denny L., Pollack A., Wright T.C.: "Policy Analysis of cervical cancer screening strategies in low-resource settings". *JAMA*, 2001, 285, 3107.
- [29] Goldie S.J., Kim J.J., Wright T.C.: "Cost-effectiveness of human papillomavirus DNA testing for cervical cancer screening in women aged 30 years or more". *Obstet. Gynecol.*, 2004, 103, 619.
- [30] Kanh L., Denny L., Pollack A., Lorincz A., Richart R.M., Wright T.C.: "Human papillomavirus DNA testing for cervical cancer screening in low-resource settings". *J. Natl. Cancer Inst.*, 2000, 17, 92, 818.
- [31] Kuhn L., Denny L., Pollack A., Lorincz A., Richart R.M., Wright T.C.: "Human papillomavirus DNA testing for cervical cancer screening in low-resource settings". *J. Natl. Cancer Inst.*, 2000, 92, 818.
- [32] Gontijo R.C., Derchain S.F., Roteli-Martins C., Braganca J.F., Sarian L.O., Morais S.S. et al.: "Human papillomavirus (HPV) infections as risk factors for cytological and histological abnormalities in baseline PAP smear-negative women followed-up for 2 years in the LAMS study". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 2006, 133, 239.
- [33] Sarian L.O., Derchain S.F., Naud P., Roteli-Martins C., Longatto-Filho A., Tatti S. et al.: "Evaluation of visual inspection with acetic acid (VIA), Lugol's iodine (VILI), cervical cytology and HPV testing as cervical screening tools in Latin America. This report refers to partial results from the LAMS (Latin American Screening) study". *J. Med. Screen.*, 2005, 12, 142.
- [34] Clavel C., Cucherousset J., Lorenzato M., Caudroy S., Nou J.M., Nazeyrollas P. et al.: "Negative human papillomavirus testing in normal smears selects a population at low risk for developing high-grade cervical lesions". *Br. J. Cancer*, 2004, 90, 1803.
- [35] Sherman M.E., Lorincz A.T., Scott D.R., Wacholder S., Castle P.E., Glass A.G. et al.: "Human Papillomavirus testing, and risk for cervical neoplasia: A 10-year Cohort Analysis". *Journal of the National Cancer Institute*, 2003, 95, 46.
- [36] Kjaer S., Hogdall E., Frederiksen K., Munk C., van den Brule A., Svare E. et al.: "The absolute risk of cervical abnormalities in high-risk human papillomavirus-positive, cytologically normal women over a 10-year period". *Cancer Res.*, 2006, 66, 10630.
- [37] Giraanelli V.R., Thuler L.C., Szklo M., Donato A., Zardo L.M., Lozana J.A. et al.: "Comparison of human papillomavirus DNA tests, liquid-based cytology and conventional cytology for the early detection of cervix uteri cancer". *Eur. J. Cancer Prev.*, 2006, 15, 504.
- [38] Herrington C.S.: "Self testing for human papillomaviruses". *J. Clin. Pathol.*, 200, 55, 408.
- [39] Nobbenhuis M.A., Helmerhorst T.J., van den Brule A.J., Rozen-daal L., Jaspars L.H., Voorhorst F.J. et al.: "Primary screening for high risk HPV by home obtained cervicovaginal lavage is an alternative screening tool for unscreened women". *J. Clin. Pathol.*, 2002, 55, 435.

Address reprint requests to:
A. LONGATTO-FILHO, M.Sc., Ph.D., PMIA
Life and Health Sciences Research Institute
School of Health Sciences, University of Minho
4710-057 Braga (Portugal)
e-mail: longatto@ecsau.uminho.pt