



## Urinary or Vaginal Self-Sampling for *Human papillomavirus* Testing in Organized Cervical Cancer Screening

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### Abstract

The poor coverage of Cervical Cancer (CC) screening in France and the insufficient performance of the cervical smear test prompt a review of screening strategies. The main objective of the PAPU-APV study is to compare two alternative approaches to CC screening, the *Human papillomavirus* (HPV) testing on self-collected First-Void Urine (FVU) and Self-Collected Vaginal (SCV) samples. The secondary objectives were to evaluate the women's preference, to assess the feasibility of self-sampling, and to determine their sensitivity and specificity to detect high-grade lesions. From April 2014 to October 2015, 461 patients were included: 176 patients from gynecological consultations (group 1) and 285 patients from the general population (group 2). Each of them performed both urinary and vaginal self-sampling. The samples were analyzed by real-time PCR and genotyping. A satisfaction questionnaire was completed to determine the best strategy to adopt between these self-sampling methods. Cervical smear test results were then collected. The prevalence of oncogenic HPV in our population was 18%, comparable in both groups. The concordance between samples was moderate (kappa coefficient  $k=0.50$ ) but there was no significant difference between vaginal and urinary samples for HR HPV detection rate (14.1% and 11.5%,  $p=0.45$ ). Urinary self-sampling was preferred over vaginal self-sampling (40.5% and 13.3%, respectively,  $p<0.001$ ). Sensitivity and specificity to detect high-grade lesions were not significantly different between vaginal and urinary self-sampling (respectively 100% vs. 100%  $p=1$  and 67.5% vs. 63.2%  $p=0.64$ ). The lack of difference between vaginal and urinary self-sampling in terms of diagnostic performance places them both as alternatives to cervical smear tests in the screening for cervical cancer, as recommended in France by the 2014 to 2019 Cancer program.

**Keywords:** *Human papillomavirus*; Screening; Self-sampling; CC; SCV

### Introduction

According to the INVS in 2012, Invasive Cervical Cancer (CC) is the 11<sup>th</sup> most common cancer in women in metropolitan France with 3,028 estimated cases, and the 12<sup>th</sup> most lethal with 1,102 estimated deaths. Globally, it is the second most common cancer in women after breast cancer.

The incidence and mortality of this cancer decreased between 1980 and 2000 (respectively 22.4 compared to 10.3 deaths from invasive cervical cancer per 100,000 women per year) thanks to the implementation of cytology-based screening in many countries. However, this decline in incidence has been reduced since the early 2000s [1] and its prognosis is deteriorating (5-year survival rate of 68% in 1989/91 compared to 64% in 2001/04) although it is the only one for which both an effective screening technique and a vaccine against the main risk factor, the *Human papillomavirus* (HPV), are available.

In France, screening is based on the cytological examination of a cervical smear test performed on a voluntary basis every 3 years. With more than 6 million cervical smear tests performed each

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year, all French women should be screened. In fact, less than 20% follow the recommendations and 40% of screened women undergo a cervical smear test every year. According to ANAES in 2003, the coverage rate in France is estimated at 60% of women. Furthermore, 40% of women diagnosed with invasive cervical cancer have never had a cervical smear test. Indeed, there are many obstacles to cervical smear testing, including gynecological examination, which patients experience as highly intrusive.

It is now well established that persistent High-Risk Oncogenic *Human papillomavirus* (HR-HPV) infection in the genital mucosa is a required factor in the onset of invasive cervical cancer [2-5]. Numerous studies have shown the relevance of testing for HR-HPV for the detection of cervical lesions [6,7], particularly on self-sampling (vaginal, urinary, vulvar), which could improve the screening coverage rate. Thus, recent French recommendation includes HR-HPV testing in primary cervical cancer screening [8].

The objective of the PapU-APV study was to compare two alternative approaches to screening for invasive cervical cancer, using First-Void Urine (FVU) and Self-Collected Vaginal (SCV) sampling to facilitate access to HPV testing. These two methods have never been compared to one another. This study is part of the evaluation of new screening strategies using self-sampling by the French National Cancer Institute.

## Materials and Methods

### Type of study

The PapU-APV study is a prospective, multi-centers, comparative, non-randomized pilot study promoted by the University Hospital of Brest. This study was validated by the French Ligue against Cancer on October 2013, the National Drug Safety Agency (ANSM) on December 2013, by the Committee for the Protection of Persons (CPP) on April 2014 and by the National Commission for Information Technology and Liberties (CNIL) on August 2014.

### Inclusion of patients

Participation in the study was systematically offered to all patients attending gynecology consultations at the Brest University Hospital, as well as at Landerneau and Carhaix hospitals. This group was called Group 1 (Gynecological follow-up). A second group was made up of women who went to the CHRU for any reason other than gynecology consultations (patient visits, dermatology consultations, endocrinology, outpatient surgery, internal medicine, pneumology, oncology consultations, and medical or paramedical staff at the CHRU). This group was referred to as Group 2 (general population).

The inclusion criteria were: To be a female volunteer affiliated to social security, aged 25 to 65 years.

The exclusion criteria were: Women over the age limit, refusal to participate, patient who had undergone hysterectomy, pregnant woman, patients who were not affiliated to the social security system.

### Self-sampling and HPV testing

Each patient who agreed to participate in the study completed a consent form and a satisfaction questionnaire. At the same time, they were given a kit containing two self-collecting samples, with instructions for use for each of the two methods.

For urine self-sampling, a Vacutainer<sup>®</sup> Cytobacteriological Urine Examination tube (CBUE) with a cannula from BD No. 364944 (with sodium borate preservative) is provided; for vaginal self-sampling,

a swab of flocked nylon No. 5298 from Copan without medium is provided.

Samples are taken on site by the patients according to the instructions for use and in the following order: Rubbing by rotation five times of the vaginal mucosa at about 5 cm from the vulva and then collecting the first urine stream in a sterile tube transferred by aspiration through the cannula in the CBUE tube. These samples are then sent, with signed consent and completed questionnaire, to the virology laboratory of the University Hospital of Brest for HPV testing. An inclusion number is then defined for the anonymization of their data recorded on an Excel database.

It is a quantitative real-time PCR test, developed specifically for the analysis of first-void urine. From a volume of 1 mL of urine and 0.2 mL of the vaginal swab in physiological buffer, viral DNA is extracted using the EasyMag<sup>®</sup> system (BioMerieux, Lyon, France) to process 24 samples in 40 min. The extracted DNA is amplified in the *L1* gene by the LightCycler 2.0<sup>®</sup> system (Roche Diagnostic, Meylan, France) in the presence of SYBR Green<sup>®</sup> to detect amplified strands whose specificity is automatically confirmed at the end of PCR by fusion curve analysis (target fusion temperature = 74°C ± 2°C). This method combines the specific research for HPV16 with a specific probe during PCR. This system allows 32 samples to be analyzed at the same time. Quantification is made possible by calibration with a SiHA cell DNA extract containing 1 copy of HPV16 DNA per cell. An internal control is included in the PCR to validate the results (DICO, Argene, France). In case of a positive test, molecular typing is performed with the InnoLIPA<sup>®</sup> system (Innogenetics, Ghent, Belgium) allowing, in 2 h 30 min, a direct hybridization of 32 genotypes from amplification products, including all HPV-HR (Class 1 : 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59), some intermediate, probable high-risk HPVs (Class 2A: 68 and 2B: 26, 53, 66, 70, 73, 82) and some low-risk HPVs (Class 3: 6, 11, 40, 42, 43, 44, 54, 61, 62, 67, 81, 83, 89).

For both urinary and vaginal samples, we searched for: the presence or absence of HPV, its concentration in Log copies of DNA/mL, the presence or absence of HPV16, as well as more precise genotyping if HPV was detected. Patients with positive HPV tests were encouraged, on the test report, to contact their gynecologist or general practitioner to obtain a cervical smear test if it had not been performed within the last 3 years, in order to meet current recommendations for CC screening. The majority of women with a negative test were reassured, with a proposal for follow-up at 3 years. In a second step, we looked for the results of each patient's last cervical smear test (call to the patient, her general practitioner, her pathology laboratory).

### Statistics

The inclusion number, the age of the patients, their recruitment method (group 1 or 2), the results of HPV tests (FVU and SCV), genotyping, cervical smear test (performed within 3 years before the HPV test or within 6 months after), and possibly the histology report were registered in an Excel file database.

The analysis of the main endpoint, i.e. the concordance between the results of the 2 tests (FVU and SCV), was estimated in each group and over the entire sample using Cohen's Kappa coefficient, with a 95% confidence interval. A test value greater than 0.8 is considered excellent, and acceptable between 0.4 and 0.8.

Diagnostic performances such as sensitivity, specificity, Positive (PPV) and Negative (NPV) predictive values, positive and negative

**Table 1:** Characteristics of patients in the study.

	Total	Group 1	Group 2	p value
Number	461	176	285	0.142 <sup>s</sup>
Age (years old; range)	41.7 ± 10.9	42.7 ± 11.2	41.2 ± 10.7	
Modes of inclusion (n, %):				
Gynaecological follow-up	176 (38.2)	176 (100)	0 (0)	
Other consultants	107 (23.2)	0 (0)	107 (37.5)	
Visitors	117 (25.4)	0 (0)	117 (41.1)	
Employees CHRU	61 (13.2)	0 (0)	61 (21.4)	
Smears performed (n, %):				
Smear test <3 years	382 (82.9)	160 (90.9)	228 (80.1)	0.004
Post-HPV smear test	303 (65.7)	134 (76.1)	169 (59.3)	0.002
ASCUS smear test (°)	8 (2.1)	3 (1.9)	5 (2.2)	0.254
LSIL Smear test (°)	13 (3.4)	7 (4.4)	6 (2.6)	0.062
HSIL Smear test (°)	4 (1.0)	1 (0.6)	3 (1.3)	0.089
HPV testing and typing (n, %):				
Positive HPV	172 (37.3)	67 (38.1)	105 (36.8)	0.362
HR genotype (°)	58 (33.7)	25 (37.3)	33 (31.4)	0.241
Genotype 16 (°)	25 (14.5)	11 (16.4)	14 (13.3)	0.123
Genotype 18 (°)	15 (8.7)	10 (14.9)	5 (4.8)	0.011

likelihood ratios (LR+ and LR-) of the two test tests (FVU and SCV) were calculated and compared in reference to cervical cytology (high grade lesions), in Groups 1 and 2, by calculating the ratio of values obtained for both tests in relation to smear tests, with a 95% confidence interval. In order to obtain sufficient precision in estimating the sensitivity and specificity ratios of the two tests (FVU and SCV), as well as in estimating the Kappa coefficient measuring the concordance between these two tests, the number of subjects required was 160 (Group 1) and 300 women (Group 2) respectively (460 women in total).

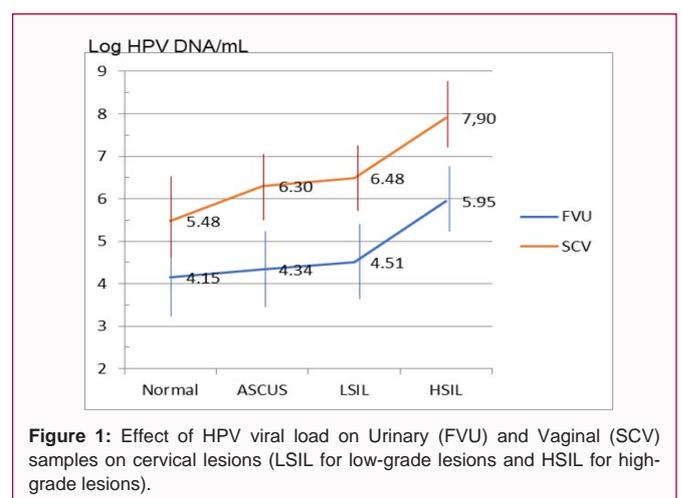
The statistical analyses were performed with SAS software version 9.4. The tests were performed considering a type I error of 0.05. A p-value less than 0.05 are considered as statistically significant, and above this threshold as statistically not significant.

## Results

### Characteristics of the study population

From April 2014 to October 2015, among 473 invited women, 461 patients were included in the study (Table 1). One patient was excluded for incorrect inclusion (over 65 years of age) and 6 patients had missing data for the primary endpoint (absence of one of the two samples). We observed only 5 refusals to participate in this study (1.06%) showing a very good participation.

One hundred and seventy-six patients were included in Group 1 (gynecological follow-up) and 285 in Group 2 (general population). The distribution of group 2, the most heterogeneous but having in common that they did not come for programmed gynecological follow-up, is represented in Table 1. Among the other consultants (37.5% of group 2), are represented women who were seen in gynecological emergencies (n=18) or who were attending consultations for infertility (n=4), contraception (n=33), internal medicine (n=26), endocrinology (n=10), dermatology (n=9) or with their child in pediatric consultations (n=7).



**Figure 1:** Effect of HPV viral load on Urinary (FVU) and Vaginal (SCV) samples on cervical lesions (LSIL for low-grade lesions and HSIL for high-grade lesions).

The average age of the women included was 42.7 ± 11.2 years in Group 1 and 41.2 ± 10.7 years in Group 2 (0.142). The most represented age groups were equally divided between those aged 30 to 39 and over 50, each representing 29.1% of the total population. The least represented age group was under 30 years of age (15.8%).

Cervical smear test in the period of less than 3 years at inclusion was obtained for 382 patients or 82.9% of the population (Table 1). Of these, 303 (65.7%) had an interpretable HPV post-test control smear test. Two patients (0.4%) had a smear test less than 3 years old with unsatisfactory interpretation. Group 1 patients had received more cervical smear tests in this period than Group 2 patients, respectively 90.9% vs. 80.1% (p=0.004). Of these cervical smear tests, 11 (6.9%) were pathological in Group 1 compared to 14 (6.1%) in Group 2 (0.125).

The HPV test was positive in 172 patients (37.3%) with all genotypes combined on at least one or both of the 2 samples (Table

**Table 2:** HPV tests concordance on FVU and SCV (n=461).

			SCV		Kappa de Cohen (95% CI)
			Negative	Positive	
	<b>Total</b>	Negative	290 (62.9%)	40 (8.7%)	0.50 (0.40-0.58)
	<b>n=461</b>	Positive	51 (11.1%)	80 (17.3%)	
<b>FVU</b>	<b>Group 1</b>	Negative	109 (61.9%)	15 (8.5%)	0.53 (0.39-0.66)
	<b>n=176</b>	Positive	19 (10.8%)	33 (18.8%)	
	<b>Group 2</b>	Negative	181 (63.5%)	25 (8.8%)	0.47 (0.35-0.58)
	<b>n=285</b>	Positive	32 (11.2%)	47 (16.5%)	

**Table 3:** Diagnostic Performance of FVU and SCV Tests to Identify High-Grade Lesions (HSIL) (n=303).

HPV Test		Negative	Cytology test HSIL	Positive
FVU	Positive	110 (36.3%)		4 (13.2%)
	Negative	189 (62.4%)		0 (0%)
	Sensitivity		1.00 (0.40; 1.00)	
	Specificity		0.63 (0.57; 0.69)	
	PPV		0.04 (0; 0.09)	
	NPV		1.00 (0.98; 1.00)	
SCV	Positive	97 (32.0%)		4 (13.2%)
	Negative	202 (66.7%)		0 (0%)
	Sensitivity		1.00 (0.40; 1.00)	
	Specificity		0.68 (0.62; 0.73)	
	PPV		0.04 (0.01; 0.10)	
	NPV		1.00 (0.98; 1.00)	
Sensitivity ratio (FVU/SCV)			1.00 (0.43; 1.32) p=0.321	
Specificity ratio (FVU/SCV)			1.03 (0.89; 1.21) p=0.642	
PPV ratio (FVU/SCV)			1.00 (0.43; 1.51) p=0.496	
NPV ratio (FVU/SCV)			1.00 (0.71; 2.31) p=0.402	

PPV: Positive Predictive Value; NPV: Negative Predictive Value

1). The prevalence of HPV in groups 1 and 2 was comparable with 38.1% and 36.8% (0.362) respectively. However, HPV prevalence varied with the age group of the patients. Indeed, the age group most infected by HPV, regardless of genotype and type of sampling or affiliation group, is under 30 years of age (41.1%) followed by 30 years to 39 years of age on an equal footing with those over 50 years of age (37.3%) and finally 40 to 49 years of age (35.0%).

The identification of at least one genotype, all samples combined, was achieved in 119 patients, representing 69.2% of HPV-positive patients. In our overall population, 13.9% of women (64/461) had HR-HPV (class 1 of the IARC classification) with 14.8% and 13.3% respectively for groups 1 and 2 (p=0.8). An oncogenic HPV (classes 1, 2A and 2B) was found in 18.0% (83/461) of the population (19.3% and 17.2% respectively for groups 1 and 2, p=0.711). The prevalence of HR-HPV is also different according to age groups (20.5% before 30 years vs. 9.1% after 50 years).

The average concentration was at 4.11 HPV DNA log/mL (± 1.77) in FVU samples and 5.63 log/mL (± 2.03) in SCV samples. The average viral load when HPV-16 or 18 was present was higher in FVU and SCV samples (respectively 4.72 and 6.71 log/mL). The concentration of HPV in log increased with the severity of cytological lesions found on the smear tests performed within 6 months after HPV testing, regardless of the type of sampling method, FVU or SCV (Figure 1).

**Acceptability of urinary and vaginal self-sampling**

Participants (n=461) felt that they were well informed about CC screening at 24.6% in Group 1 and 28.6% in Group 2 (p=0.35). Of these, 99.8% found the self-sampling approach (FVU and SCV) simple to implement and 100% would recommend doing this test.

In the overall population, no preference was found for FVU or SCV in 46.2% of participants (54.3% in Group 1 and 41.1% in Group 2), a preference for FVU was 40.5% and for SCV 13.3% (p<0.001). In the subgroup study, only patients in group 2, who had no gynecology follow-up, significantly preferred FVU (48.6% preference for FVU vs. 10.3% for SCV with p<0.001), while in group 1, the preference for FVU was only 27.4% vs. 18.3% for SCV (p=0.056).

**Comparison of HPV tests on urinary and vaginal samples**

In total, the results (positive or negative for HPV) were concordant between the 2 tests for 370 patients (80.2% of the total population) with 80 concordant positive tests (17.3%) and 290 concordant negative tests (62.9%). Cohen's kappa coefficient was acceptable at 0.50 and comparable in each of the 2 groups (Table 2).

For concordant positive HPV tests, genotype identification was perfectly identical for 41 sample pairs (51.9% of concordant positive tests) and 17 multi genotype samples (21.5% of concordant positive tests) were concordant for at least one genotype. In total, this represents 58 matching samples for at least one genotype (73.4%

**Table 4:** Improvement of diagnostic performance of high-risk lesions (HSIL, n=4).

Condition	Selection	% HSIL	Fold-improved
If no test	Study population	0.90%	1
if HPV + test	FVU +	3.10%	3.5
	SCV +	3.30%	3.8
if HPV + HR test	FVU +	8.50%	9.8
	SCV +	7.00%	8.1
if HR-HPV + 16/18	FVU +	15.40%	17.7
	SCV +	13.30%	15.4
if HR-HPV + 16/18 and high viral load	FVU + >4 log	23.50%	27.1
	SCV + >6 log	23.50%	27.1

of matching positive tests). Seven matching positive samples were different in terms of genotype (8.9%) and 14 matching positive tests could not obtain genotype identification despite a second PCR analysis (17.7% of the matching positive tests).

### Diagnostic performance

Since HPV and HR-HPV prevalence were equivalent in groups 1 and 2, they were combined to increase the number of post-HPV test smears for which results were recovered (n=303, or 67.5% of the population in both groups) and the number of high-grade lesions detected (n=4, 1 in group 1 and 3 in group 2). As found in Table 3, FVU and SCV tests are not significantly different in terms of sensitivity, specificity, PPV and NPV, and positive and negative likelihood ratios, as shown by the FVU/SCV ratios in which p values are all higher than 0.05. In our study, 100% of high-grade lesions (n=4) are found with a positive HPV test on urinary samples and vaginal samples, with 3 HPV16 and one HPV18 matching.

The diagnostic performance of high-grade lesions can be improved by combining the available viral markers, with a probability ranging from 0.9%, in the absence of a test, to 23.5% if the HPV+ test on SCV or FVU is also HR+ with a HPV16 or 18 genotype and a high viral load, which is 27 times higher than the performance without a test (Table 4).

## Discussion

### Assessment of the screening situation and limitations of the cervical smear test

Current screening based on the cervical smear test could be improved. We have shown in our study that the use of vaginal and first-void urinary self-sampling for high-risk HPV testing at similar performance levels could help to improve the performance (up to 27 times) of cervical cancer screening to identify high-grade lesions in general population.

Indeed, the cervical smear test has a low sensitivity for a screening test. In his meta-analysis of 62 studies, Fahey et al. found an average sensitivity for the cervical smear test of 58%, improved by the routine repetition of cervical smear tests, which increases the cost of screening [6]. Moreover, the sensitivity of the cervical smear test appears to be highly age-dependent, with a much better sensitivity after 50 years (79.3%) than in younger women (59.6%), even though the peak incidence of high-grade lesions (CIN2+) is now less than 35 years old and the peak incidence of invasive cancer is 41 years old [7]. In addition, the development of vaccination will decrease the positive predictive value of cytology, causing it to lose diagnostic value again, making it insufficient to control vaccinated women.

Finally, the cervical smear test does not provide exhaustive coverage of the population because of the many obstacles related to the patient, access to care, but also to the type of sample requiring a gynecological examination. The organization of screening at national level by using invitation and reminder methods for cervical smear tests could improve the rate of participation in screening, but some obstacles to screening will still be present (access to care and method of sampling) [9].

Given these limitations, self-sampling seems more appropriate. Many studies have shown that self-sampling significantly improves the participation rate compared to a sample taken by a clinician [10-12]. Piana et al. [13] found a 7.2% participation rate following a second invitation to perform a cervical smear test vs. a 26.4% participation rate in the group of patients who were invited to perform vaginal self-sampling (p<0.001) [13]. The 5-year follow-up of patients in the PapU29 cohort showed a 3.7% participation in the cervical smear test group compared to 28.2% in the first-void urine self-sampling group, i.e. a 7.6-fold increase in the participation rate for urine self-sampling [14].

It is therefore important to develop techniques and to improve screening coverage while improving the inherent characteristics of the proposed tests.

### Contribution of self-samples

Regarding the clinical impact of self-sampling, Lazcano-Ponce et al. [15] compared the detection of HR-HPV on vaginal samples with the cytological result of cervical smear tests in Mexican women aged 25 to 65 years of age living in precarious socioeconomic conditions. Their study shows that the relative sensitivity of CIN2+ detection of the HR-HPV test on vaginal sample was 3 to 4 times greater than that of the cervical smear test [15]. Stanczuk et al. [16], found HPV sensitivity on vaginal samples of 94.6% for the detection of CIN2+ lesions with a specificity of 85.4%, figures similar to those observed in the HPV test on cervical samples taken by a clinician [16]. These figures, however, seem less convincing when it comes to low-grade lesions. Indeed, De Alba et al. [7], found a similar sensitivity between vaginal samples and cervical smear tests to identify lesions of low grade or higher (55% vs. 50%, p=0.45), but less specificity (79% vs. 94%) [7].

In its meta-analysis, Arbyn et al. [14] observed an increasing participation of more than 75% of targeted patients in screening through the proposal of vaginal self-sampling, now applied in the Netherlands to non-respondent women [14]. Vaginal self-sampling therefore appears to be useful for the detection of oncogenic HPV in the catching-up of undetected patients. Indeed, in their meta-

analysis, Ogilvie et al. [17] found a Cohen Kappa's coefficient for the HPV test performed on cervical smears obtained by a clinician *vs.* that on vaginal self-sampling between 0.45 and 1. His conclusion is that vaginal self-sampling can be used to screen patients who are not undergoing gynecological follow-up or in developing countries [17]. For this purpose, vaginal self-sampling should be combined with HPV testing based on PCR.

Indeed, according to a previous meta-analysis by Arbyn et al. [14] in 2014, the only studies with a high level of concordance were all based on PCR techniques. They found that vaginal self-sampling is significantly less sensitive and specific than the cervical smear test for the detection of HR-HPV when samples were analyzed by signal amplification techniques (Hybrid Capture II type). However, this problem seems to be improved when a target amplification technique (PCR) is used and it is therefore the technique that should be proposed in the context of HPV research on vaginal samples [18].

In France, a team from Tours in the Apache study, comparing a HPV test performed on a vaginal sample and a HPV test in a liquid medium collected by a clinician, found a Cohen's Kappa coefficient at 0.76 and a concordance rate at 91.7%, with a sensitivity and specificity of 88.7% and 92.5% respectively for HPV detection [19]. Simultaneously, we participated in a French multicenter PapU study evaluating the first-void urine self-sampling HPV test compared to the liquid HPV test performed by the clinician showing a concordance of 92.1% and a Kappa coefficient of 0.83, with a sensitivity of 95.2% and a specificity of 62.3% to identify high-grade lesions, comparable to those obtained with the HPV cervical smear test [20].

The detection of HPV in urine specimens was initiated by Das et al. [21] in 1992. He observed that in 19 patients with cervical dysplasia or cervical cancer, the search for HPV-16 at the urinary level is concordant in 17 cases (positive or negative) and 2 cases found HPV-16 in the urine but not on cervical samples. This observation paved the way for further studies [21]. In this review of the literature, the HPV detection capability appears to be better in cervical specimens than in urine samples. One explanation is that the viral load found in urinary self-samples is 50 times lower than in cervical samples, a phenomenon that is circumvented by the use of DNA amplification techniques and the adaptation of extraction methods to large volumes of urine [22].

A systematic review with a meta-analysis published in 2014 by Pathak et al. [23] found a sensitivity of 77% (from 68% to 84%) and a specificity of 88% (from 58% to 97%) for the detection of HR-HPV in urine. More specifically, they found a slightly lower combined sensitivity for the detection of HPV-16 and 18 in urine samples of 73% (from 56% to 86%) and a higher specificity of 98% (from 91% to 100%), even though these two types of HPV are responsible for 70% of cancers. In this meta-analysis, the studies are quite heterogeneous with regard to urine collection techniques and in particular the tests carried out on the first-void urine seem to offer better performance than those carried out in mid-stream urine or random urine (sensitivity increases from 74% to 89% between mid-stream and first-void urine). The urinary test is also of significant interest since there is a high specificity compared to the cervical smear test with a positive likelihood ratio of 15 for all HPV types (with a ratio of 37 for HPV-16 or HPV-18), thus avoiding a significant number of false positives. In our study, with a method based on quantitative real-time PCR research, adapted for first-void urine self-sampling we obtain results comparable to those on vaginal self-sampling, with a patient

preference for urine self-sampling [22].

### Acceptability of methods

The self-sampling method satisfies patients up to 95% [24], and is often preferred over vaginal sampling performed by the clinician [25]. In the comparative study by Sellors et al. [26] in 2000, 98.4% of patients found urinary self-sampling acceptable, 92.9% for vulvar sampling, and 88.2% for vaginal sampling [26]. In our study, the majority of patients (46.2%) did not have a preference between the two tests. However, in line with Sellors' findings and more in line with the target population for self-sampling, the majority of patients in Group 2, who were not undergoing follow-up, had a significant preference for urine self-sampling, while those with regular gynecological follow-up (Group 1) had greater confidence in vaginal sampling, while remaining below the preference's rate for urine self-sampling.

### Self-sampling performance to identify high-grade lesions

Few studies have specifically compared the performance of urinary versus vaginal self-sampling. Nicolau et al. [27] in a population of 75 patients with CIN2+ lesions, found an HPV prevalence of 58.8% in CIN2 and 78.0% in CIN3. According to them, HPV tests performed on urine samples have a lower sensitivity and specificity than those performed on cervical specimens or on other self-collection methods such as vulvar or vaginal samples [27]. These elements are corroborated by the study by Sellors et al. [26], which found that the detection rate of HPV (in patients with high-grade lesions) decreases with distance from the cervix: compared to a cervical sample collected by a clinician, the detection's sensitivity of HR-HPV for vaginal samples is 86.2%, that of a vulva samples is 62.1% and that of urinary samples is 44%. This phenomenon is also found by Sahasrabudhe et al. [28] who found a detection rate of HR-HPV in a urine sample of 58.3% compared to 73.6% in a cervical sample taken by a clinician and 72.1% for a vulvar sample [28].

The Stanczuk study compares the clinical performance of urinary and vaginal self-sampling as in our study and finds sensitivity and specificity of 63.1% and 89.8% respectively for urinary self-sampling *vs.* 94.6 and 85.4% for vaginal self-sampling. However, the urine was collected randomly, reducing the sensitivity of the test [16].

Only the results of Leeman et al. [29] do not show any significant difference in the detection of CIN2+ lesions between the HPV test performed on cervical smear tests, urine self-sampling (on first-void urine) and vaginal self-sampling with sensitivities of 96.4%, 96.4% and 92.9% respectively.

Although the clinical performance of self-sampling is difficult to interpret in our study due to insufficient cytopathological data available to observe high-grade lesions ( $n=4$  out of 303), there does not appear to be any major difference between the two methods of self-sampling in terms of ability to detect high-grade cervical lesions, consistent with their comparable analytical performance.

One way to improve the diagnostic performance of these samplings and the specificity of screening would be to establish a prognostic score combining HR-HPV testing, genotyping, viral load and cervical cancer biomarkers. In our study, this performance is improved by 3.5 times with HPV testing and by 27 times if associated with genotyping and viral load. It could be further improved with the addition of one or more high-grade lesion biomarkers, as found in urine self-sampling [25].

## Analysis of discrepancies

In our study we show a two-way discrepancy (FVU+/SCV- of 11.1% and FVU-/SCV+ of 8.7%), with a total of 19.8% discrepancy. Discrepancies associated with negative vaginal self-sampling may be due to improper sampling. Indeed, it is important to rub the vaginal mucosa thoroughly to collect the cells present on the mucosa and possibly carrying HPV. A false positive cannot be excluded from the urine self-sampling or from another anatomical site (e. g. anal). Discrepancies associated with negative urinary self-sampling may also be due to the absence of cells, particularly with a mid-stream sample as reported in the meta-analysis of Pathak et al. [23]. Unfortunately, most tests marketed for HPV testing do not contain cellular gene controls to support these hypotheses. This control should be available for the implementation of HPV tests on self-sampling for their validation.

## Study limits

Regarding the performance of each of the tests, our results indicate excellent sensitivity and adequate specificity, comparable for both approaches. But these results should be taken with caution as most post-HPV cervical smear tests were taken within 6 months of the test, only 90 cervical smear tests (29%) were performed on the same day. An interesting approach would have been to obtain a liquid cervical smear for all patients included on the day of the self-sampling in order to have, on the one hand, a recent cytological analysis to calculate the performance of the HPV test and, on the other hand, to be able to carry out an HPV test with a sample taken using a technique that is approved, such as the performance of a cervical smear test carried out by a professional. There is also a population selection bias between the two groups since in group 2, being already resistant to conventional screening; it seems difficult to obtain both self-sampling and cervical smear control for this group.

## Conclusion

Individual cervical smear screening remains insufficient, prompting the scientific community to look for other tools for secondary prevention of cervical cancer. The organization of screening could be an answer, but the many obstacles to carry out a gynecological examination limit its impact. In addition, HPV vaccination should undoubtedly reduce the prevalence of HPV leading to a decrease in cervical smear's positive predictive value. Thanks to better adherence, HPV testing from self-sampling is therefore attractive.

In order to be able to properly compare studies, particular attention must be paid to the standardization of sampling and analysis techniques. Indeed, to date, in view of the data in the literature, whose protocols may be very different, it seems reasonable to propose our approach only to women who do not respond to organized cervical cancer screening.

In our study, first-void urine HPV test is therefore easy to perform and preferred by unmonitored women, who are targeted for screening, with comparable performance to the HPV test on vaginal self-sampling, which has been widely evaluated in the literature.

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