Urinary HPV DNA testing as a tool for cervical cancer screening in women who are reluctant to have a Pap smear in France

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Summary

Objectives: In France, cervical cancer screening is based on human papillomavirus (HPV) testing on cervical samples (women aged 30-65) and cytological examination of Pap smears (25-29), but screening coverage is unsatisfactory. The CapU3 study aimed to propose urinary HPV testing on 13,535 women aged 35 to 65 who had not had a Pap smear since 2010.

Methods: High-risk HPV (HR-HPV) detection was performed using a real-time PCR (Anyplex II HPV 28 Detection, Seegene®). Women with HR-HPV positive results were encouraged to have a cervical smear as soon as possible to detect the presence of cervical lesions.

Results: The participation rate was 15.4%. Out of the 1,915 analyzed specimens, 1,711 and 190 were negative and positive, respectively, for at least 1 HR-HPV genotype. HR-HPV genotypes other than HPV-16 or HPV-18 were mostly detected as HPV-53 (23.7%) and HPV-68 (14.2%). A satisfactory gynecological follow-up was observed for HPV-positive women (92.1%). 23 abnormal smears were observed and eight high-grade cytological lesions after colposcopy and biopsy were diagnosed.

Conclusions: As home HPV urinary testing is non-invasive and does not require medical attention, this method may be an alternative for women who are reluctant to have a Pap smear and thus extend screening coverage.

Keywords: High-risk human papillomavirus; Urine; HPV DNA detection; cervical cancer screening; Self-sampling

Introduction

In 2018, cervical cancer was the 4th most common cancer in women for incidence and mortality after breast, colorectal, and lung cancer (1). In France, in 2017, it was the 12th most common cancer with 2,800 new cases each year. Regarding mortality rate, it is 4th in the world (about 270,000 deaths per year), 1st in low-income countries, and 12th in France (about 1,100 deaths per year) (2,3).

Cervical cancer screening over the last 50 years in Europe has significantly reduced the incidence and mortality associated with cervical cancer. In France, between 1980 and 2019 (June), cervical screening was done through cytological examination of cervical-uterine samples (Pap smear) in women aged 25 to 65 (4). However, at this time, despite the campaigns and letters of encouragement to have a Pap smear, the screening coverage remained low (below 60%) (2). Vaccination coverage rate against HPV remained also low (<30%) (5) in view of the objectives planned at 60% by the 2014-2019 Cancer Plan (6) and is one of the lowest rates in Europe.

Therefore, reflection tracks are being considered to improve screening coverage. High-risk human papillomavirus (HR-HPV) testing on self-samples could be one way to increase access to cervical cancer screening for women not participating in routine screening. Since July 2019, the French National Health Authority (*Haute Autorité de Santé*, HAS) has updated the recommendations for cervical cancer screening and proposed a national screening strategy that includes HPV testing. For women aged 30 to 65, cervical cancer screening is based on HPV testing of cervical samples every five years. For women aged 25 to 29, cervical cancer screening is based on cytological examination of a Pap smear every three years after two consecutive annual smears (7).

Other options are envisaged in order to increase screening coverage, notably research into HPV through self-sampling. This includes, in particular, urinary self-sampling, which has the advantages of being less invasive and/or often better accepted than the Pap smear. Arbyn et al. recommended HPV testing on self-collected samples as an additional strategy to reach women who do not participate in regular screening programs (8). Indeed, self-sampling could be an alternative for women who are reluctant to have a Pap smear and thus allow extending the cervical cancer screening coverage. The urinary self-sampling could be a preferred option for these women because it is understandable, non-invasive, and easy to do themselves. However, the topic is quite controversial (9–11) as urine is a complex biological medium with many inhibitors and HPV DNA can be destroyed by bacteria or endonuclease. Nevertheless, this type of self-sampling, which has recently been introduced, is increasingly proving its worth in clinical studies (12–14). Detection of HPV DNA in first-stream urine is recommended due to a large amount of DNA (15) and a high quantity of exfoliated cervical cells (13) in the urine. In 2014, a meta-analysis was published by Pathak and his collaborators summarizing the 14 studies on the detection of HPV DNA in urine. The authors showed that urine is highly effective in HPV DNA detection, and this type of sampling could be an acceptable alternative in populations with little or no cervical screening (14).

In 2015, we reported the results of our CapU1 study, which offered urinary HPV testing to 5,000 women aged 45 to 65 years. We showed that urinary HPV testing may be pertinent to women who had not had cervical Pap smears in three years. In doing so, the CapU1 study led to the diagnosis of high-grade cervical lesions (three cases of cervical intraepithelial neoplasia grade III lesions confirmed, CIN 3) (12).

The objectives of the CapU3 study were to (i) make women who are very reluctant aware of cervical cancer screening, (ii) assess the participation rate of urinary HPV testing on a larger

number of women and a broader age range than previous works, and (iii) increase cervical cancer screening coverage. Consequently, the aim of the study was to propose an alternative screening method for cervical cancer to women who had not had a gynecological follow-up over the past seven years and to reintroduce these women to gynecological screening and follow-up.

Material and Methods

Study design

The CapU3 study was conducted in the Maine et Loire department (Pays de la Loire, France). This study was a collaboration between the Regional Cancer Screening Coordination Center (CRCDC, formerly Cap Santé 49) and Angers University Hospital. CRCDC, which is a public health body governed by the French law on non-profit associations, provides colorectal, breast, and cervical cancer screenings in this department. Thus, since 2010, CRCDC has sent letters to women aged 25 to 65 who have not had a Pap smear over the past three years. Secondary reminders after nine months were sent if CRCDC did not receive a Pap smear report or observe a reimbursement for a Pap smear from the national health insurance system. The list of patients acquired by CRCDC from the health insurance companies included first and last names, dates of birth, addresses, and social security numbers in order to avoid confusion and duplications. The supply and use of these records for the program was approved by CNIL (*Commission nationale de l'informatique et des libertés,* French National Data Protection Authority) with authorization number 1410571.

Between November 2016 and November 2018, 13,535 women aged 35 to 65 received a fifth or sixth letter proposing a new screening method based on self-sampling urine instead of a Pap smear. Their mean age was 54 years. These women had not had a Pap smear over the past

seven years and did not respond to four or five reminders. With the letter, the women received an information note on urinary HPV DNA testing, a letter of consent, a sterile container, a sampling protocol, a document to ask the women if they had had a recent cervical smear or hysterectomy, a bubble envelope, and a prepaid return envelope. The women noted the name of their physicians or gynecologists on the letter of consent. Women accepting to participate had to send their first-stream urine samples by mail to the Angers University Hospital Virology Laboratory using the bubble envelope and the prepaid envelope in accordance with a three-rule secure packaging protocol as recommended in France. The mailing of the CapU3 study package was managed by a business solutions unit of the national postal service to optimize the process. Notably, packages that could not be delivered to their addressees (the person had moved, etc.) were recorded by the business solutions unit.

A statement of collection was made by the relevant ethics committee in compliance with existing regulations (authorization number 2016/102).

Response rate and participation rate

The number of non-inclusions, corresponding to invitation letters that did not reach the women (not living at the stated address, referral, or damaged mailboxes), has been subtracted from the total number of letters sent. The response rate was based on the receipt of prepaid return envelopes (with or without the urine sample) at the virology laboratory. The response rate was calculated as follows: *Number of letters received at the laboratory / (Number of letters sent to women - Number of non-inclusion)*

The participation rate was determined based on the receipt of accepted informed consent forms and urine samples. Women who sent their urine samples but had a history of hysterectomy or recently had a Pap smear (before receiving the letter) were excluded. Women who sent their urine samples without signing the letter of consent were contacted again to

confirm that they accepted to be included in the study. Some women sent their urine samples but did not want to participate in the study; they were thus considered ineligible for the study and not counted in the participation rate.

The participation rate was determined as follows: *Number of women included in the study / (Number of letters sent to women - Number of exclusions and non-inclusion)*

Virological analysis

HR-HPV detection was performed with Anyplex™ II HPV28 Detection technology (Seegene®) using real-time PCR. This technique is based on TOCE™ (Tagging Oligonucleotide Cleavage and Extension) technology that can detect multiple pathogens in a single fluorescence channel on real-time PCR instruments. This is a combined test for detecting HPV DNA and genotyping 28 HPV types, split into two panels: panel A with 14 HR-HPV genotypes (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) and panel B with five HR-HPV genotypes (HPV 26, 53, 69, 73, 82) and nine low-risk HPV genotypes (HPV 6, 11, 40, 42, 43, 44, 54, 61, 70).

Each urine sample was stored in aliquots at -20°C when received at the laboratory, awaiting HPV DNA extraction. The Seegene® method was previously evaluated on 370 urine samples at the virology laboratory of Angers University Hospital (16).

The HPV DNA was then extracted from 1 mL of urine sample using the NucliSENS® easyMAG® (Biomérieux). The HPV DNA amplification was performed according to the manufacturer's instructions and 5 µL of nucleic acids were extracted from the urine sample. The amplification of HPV DNA was performed on the CFX96 ™ thermocycler (Biorad®). Internal control (IC) amplicons were used to assess the sample validity, extraction, and amplification efficiency.

144 Samples in which at least one of the 19 HR-HPV signals were detected, whether or not it was
145 associated with the detection of an IC signal, were considered positive. Samples in which
146 none of the 19 HR-HPV signals were detected, while the IC signal was valid, were considered
147 negative. Finally, if none of the 28 HPV signals were detected and the IC signal was weak or
148 was not detected, the samples were considered invalid. The HR-HPV result included HPV
149 genotypes that are "carcinogenic to humans" (Group 1, HPV 16, 18, 31, 33, 35, 39, 45, 51, 52,
150 56, 58, 59), "probably carcinogenic to humans" (Group 2A, HPV-68), and "possibly
151 carcinogenic" (Group 2B, HPV 26, 53, 69, 73, 82) according to IARC classification (17).
152 If the HPV test was negative, the result was sent to the women and to their physicians by
153 letter. We nonetheless recommended that the women had a Pap smear in the following year.
154 In the event of a positive HPV result (one or many HR-HPV types detected), the result was
155 sent directly to the physicians and/or gynecologists. The woman was then contacted by her
156 physician and/or gynecologist to have a Pap smear as soon as possible. Women who did not
157 have a Pap smear within two months after the first positive result received another reminder
158 letter from the CRCDC.

159 ***Cervical cytology analysis***

160 Cervical cells were obtained using a cervical brush for conventional cytological slides by
161 general practitioners or gynecologists. Cytological examination was done according to the
162 consensus guidelines of the French National Health Authority and formulated according to the
163 2001 Bethesda classification (normal; atypical squamous cells of unknown significance
164 (ASC-US); atypical squamous cells - cannot exclude high-grade squamous intraepithelial
165 lesion (ASC-H); low-grade squamous intraepithelial lesion (LSIL), or high-grade squamous
166 intraepithelial lesion (HSIL)) (18). Women with cytological abnormalities underwent
167 colposcopy and biopsy as per French recommendations (*Haute Autorité de Santé*). The

168 histological results were formulated on the report according to Richard's classification: CIN
169 (cervical intraepithelial neoplasia) 1, CIN 2 or CIN 3.

170 ***Statistical analysis***

171 All statistical analyses were performed using IBM® SPSS® 15.0 Statistics (Statistical
172 Package for Social Sciences, IBM Corp., Chicago, IL) and Microsoft Excel 2010. A two-
173 proportion z-test, a Chi-square test, and a Mann-Whitney U test were used. Statistical
174 significance was considered for p values of <0.05.

175

Results

Response rate

Between November 2016 and November 2018, 13,535 letters were sent to women aged 35-65 years. Of the 13,535 letters sent, 12,958 reached their respective addressees and 577 were unsuccessful, i.e. 4.3% (not living at the stated address, referral, or damaged mailboxes). 2,394 letters with or without urine samples were received at our laboratory, i.e. 18.5% of responses.

The average response time after receipt of the self-sampling kit at home was 35 days with a median of 21 days (5 - 462 days).

Participation rate

Of these responses, 479 women were excluded from the study for the following reasons: 345 women for medical history of hysterectomy; 63 women due to recently having had a smear test; 37 women refused to participate in the study; 34 women for other reasons, such as patient residing outside the department, death, incomplete file, hospitalization, or unidentified reason (Figure 1). 1,915 women were included in the CapU3 study (letter received with the urine sample and signed consent without exclusion criteria), with a participation rate of 15.4% (Figure 1). Indeed, the participation rate was significantly different between age groups 45-49 (16.9%) and 50-54 (14.2%) ($p = 0.014$); between age groups 45-49 and 55-59 (14.1%) ($p = 0.020$); between age groups 45-49 and over 60 years old (13.4%) ($p < 0.001$). The mean age of women included was 53.8 ± 8.5 years.

HPV prevalence

Out of the 1,915 samples analyzed, 1,711 were negative and 190 were positive (detection of HR-HPV), i.e. an HR-HPV prevalence of 9.9% (95% Confidence Interval, [CI]: 8.7%-

11.3%). There were only 14 samples with no internal control amplification (0.7%) and thus HPV DNA detection could not be validated. The mean age of HPV-infected women was 54.2 ± 8.7 years and the mean age of non-HPV infected women was 53.8 ± 8.5 years.

Among the 190 HR-HPV positive samples, genotypes other than HR-HPV-16/18 were the most frequently detected ($p<0.0001$) (Table 1). HPV-16 or HPV-18 was detected in coinfection with other HR-HPV types in 16/190 or 8.4% of our patients. The mean age of women infected by HPV-16 was 58.4 ± 6.0 years, and the mean age of women with coinfection was 55.0 ± 8.5 years. The mean age of women with HR-HPV other than 16/18 was 53.4 ± 8.8 years.

Independently of single infection- or coinfection, the distribution of HR-HPV types showed that the main genotype identified was HPV-53 (23.7%) followed by HPV-68 (14.2%), HPV-16 (13.2%), and HPV-31 (12.1%). HPV-18 was detected in only 6.3% of cases (Figure 2). Distribution of HR-HPV types among HR-HPV positive women and according to IARC classification was: 131/190, 68.9% group 1; 21/190, 11.1% group 2A and 38/190, 20.0% group 2B.

Multi-strain infections with two or more HR-HPV genotypes were identified in 30.0% of cases. Single infection was diagnosed for the majority (70.0%).

Gynecological follow up, cytological and histological results

190 women were referred to their general practitioners or gynecologists for a cytology test and/or colposcopy following a positive urinary HPV test. Of the 190 HPV-positive women, 175 of them carried out a Pap smear and one woman was monitored for anal cancer (in this case, Pap smear is contraindicated for two years after radiotherapy). Therefore, the percentage of Pap smears performed after a urinary HPV test was 92.1%. The cytological results showed

148 (84.6%) satisfactory Pap smears without dysplasia, 23 (13.1%) abnormal Pap smears, two inflammatory Pap smears (1.1%), and two unsatisfactory Pap smears (1.1%). Of the 23 abnormal smears, four HSIL (17.4%), 15 LSIL (65.2%), and four ASC-US (17.4%) were diagnosed. Colposcopy with biopsy confirmed six CIN 1 lesions, eight CIN 2-3, and seven without dysplasia (Table 2). Seven CIN 3 lesions correspond to four HSIL, two LSIL, and one ASC-US. The prevalence of high-grade lesions in the present study was 8/23 abnormal smears, i.e. 35%. Despite reminders, two women refused to consult their gynecologists to have a colposcopy.

The age of women with HSIL (43.8 ± 6.0 years) was significantly different from those with normal Pap smears (53.8 ± 9.1 years; $p = 0.039$) and those with LSIL (55.4 ± 5.5; $p = 0.014$). Women with HSIL tended to be younger than those with normal Pap smears and LSIL Pap smears.

It is interesting to note that the HR-HPV genotypes implicated in high-grade cervical lesions (CIN 3 lesions) were mostly HPV-31 and HPV-51 in single infection or coinfection.

Of the 190 HR-HPV patients, 36 women (18.9%) were infected with HPV-16 and/or HPV-18 (single or coinfection with other HR-HPV types). The cytological results in these 36 women were as follows: 26 normal smears, one HSIL, four LSIL, one unsatisfactory, and four not performed. The HPV-16 genotype was associated with high-grade cervical lesions in two patients (one in single infection and one in coinfection).

Discussion

In this study, the main objective of increasing cervical cancer screening coverage in our department was largely achieved as we invited almost all women aged 35 to 65 years, i.e. 13,535 women, who had not yet had a Pap smear since 2010.

The CapU3 study has shown high levels of acceptance of urinary self-sampling by participants. We gained two participation points compared to the previous CapU1 study conducted in our department. Indeed, in the CapU3 study, we estimated a participation rate of 15.4% versus 13.7% in the CapU1 study (12), while the women invited to the CapU3 study had not had a Pap smear for at least seven years versus three years in the CapU1 study. The CapU1 study was the first to evaluate a new strategy involving HPV detection in urinary self-sampling at home, in addition to a cervical cancer screening program. In our study, it would appear that the participation rate in the 45-49 age group was higher but this result must be interpreted with caution because of a larger number of exclusions in the age group of 55 years old and above (hysterectomy).

Our overall participation rate is hopeful and similar to that recently published in Arbyn's meta-analysis, namely 19.2% (95% CI: 15.7 - 23%), knowing that our study concerns women who are very reluctant to have a Pap smear (no Pap smear over the past seven years and no response to four or five reminders to have a Pap smear). The participation rate in this meta-analysis was determined from 21 studies involving women who were not regularly screened and received a vaginal self-sampling kit at home. This meta-analysis also showed that strategies for sending self-sampling kits to patients at home or door-to-door actions are more effective than “opt-in” strategies in which women take the initiative to request a self-sampling kit or common nationally organized screening programs with invitation letters to have a cervical smear (10% of response rate) (19).

The average response time after receipt of the urinary self-sampling kit at home was 35 days with a median time of 21 days. This result shows that it is not appropriate to send reminders by mail to women within a month of receiving the first invitation letter.

In the present study, virological analysis included data on HPV prevalence, distribution of HPV genotypes and their respective impacts on the development of cervical lesions.

The prevalence of HPV infection depends on parameters including the age of patients, socio-economic level, and the presence of cervical lesions (20,21). Age is a factor to consider in order to evaluate the prevalence of HPV infection. Indeed, the first peak of prevalence is observed in young women under 25 years old, a phenomenon related to their sexual activity. The second peak is found in postmenopausal women aged over 50 years old. In the CapU3 study, we reported HPV prevalence at 9.9% in accordance with international data (20). The HPV prevalence, higher in the CapU3 than in the first CapU1 study (4.2%), may be explained by the younger age of the invited women (35-65 years in CapU3 versus 40-65 years in CapU1) and by the highest number of genotypes detected by the technique used (19 HR-HPV in CapU3 versus 14 in CapU1). Several studies have also shown that HPV prevalence in urine was higher in symptomatic women monitored for abnormal smears than in asymptomatic women participating in routine screening (22,23). In our previous work (the PapU study), high HPV prevalence, i.e. 42%, was discovered in symptomatic women who had been referred to gynecological consultations for abnormal smears. Moreover, the mean age in the PapU study was lower than in the CapU3 study (36 years old versus 54 years old) (23).

The CapU3 study confirms our previous results on the predominance of HR-HPV types other than genotypes 16/18 among the HPV cases detected, regardless of the cytological results (12). It was primarily the HPV-53 genotype detected in our target population with detection in 23.7% of HPV-positive samples followed by genotype 68 detected in 14.2%. HPV-16 and

HPV-18 represented 13.2% and 6.3%, respectively, of HPV-positive samples, regardless of the cytological results and these results were equivalent to the prevalence described in France: 15.2% for HPV-16 and 2.4% for HPV-18 (24).

Coinfection with multiple HR-HPV types was frequently detected regardless of the cytological results, as previously described in other studies (12,25).

Monitoring is frequently difficult in experimental studies. In our study, a successful gynecological follow-up after positive urinary HPV testing was obtained with a rate of 92.1% of women having a Pap smear. Planned calls to the patients' physicians or gynecologists permitted successful monitoring. Nevertheless, 15 women did not have a Pap smear after the invitation and reminders (two reminders, the last of which was a follow-up letter). Among these women, one woman was monitored for anal cancer and radiotherapy treatment, contraindicating the Pap smear for two years. One woman consulted her physician without planning a Pap smear. After the physician's call, 13 women were unknown by the physicians or gynecologists. Among women with a Pap smear result, two women refused to consult their gynecologists and were very reluctant to have a colposcopy.

Colposcopy with biopsies confirmed seven CIN 3 and one CIN 2 lesions. As previously described, the sensitivity in urine samples for detecting CIN 2+ (95% for morning first-void urine and 100% for first-void urine from later during the day) was satisfactory because it did not significantly differ to Pap smear sensitivity (100%) or sensitivity of brush-based self-sample (100%) (26).

In this study, we reported a predominance of HR-HPV types other than HPV-16/HPV-18 in abnormal smears. In the French HPV reference laboratory, HPV prevalence increased with the severity of lesions and HR-HPV genotypes other than 16/18 were more frequently

316 described in normal, ASC-US, and LSIL cervical smears (24). HPV-53 genotype was the
317 second most frequently detected genotype with HPV-66 after HPV-16 in the cervical cancer
318 screenings (24). In the CapU3 study, HPV-53 was associated with coinfection with one CIN 1
319 and one CIN 3 lesions, but it was probably not the genotype responsible for the severity of
320 these lesions. HPV-16 genotype was associated with two CIN 3 lesions: with HSIL smear in
321 one woman (1/4, 25%) and with LSIL smear in another one (1/15, 7%). The CapU3 study
322 therefore confirms the accuracy of urine samples in detecting high-grade cervical lesions. The
323 prevalence of high-grade lesions in the present study was 35%. Indeed, genotypes 31 and 51
324 were most frequently implicated in the CIN 3 lesions, suggesting a significant role of these
325 HR-HPV types that are not HPV-16/HPV-18 in oncogenic HPV persistence and progressive
326 development into severe cervical lesions. Knowing that currently available HPV vaccines do
327 not protect against HPV-51, the emergence of this genotype in the future could have an
328 impact on public health.
329
330 No HPV assay is currently marked for first-void urine. However, Pathak et al. demonstrated
331 that the sensitivity and specificity of HPV DNA detection in urine samples (by PCR for most
332 methods) were satisfactory (87% and 94% respectively) and that testing first-void urine
333 samples was more accurate than random or midstream sampling (14). Nevertheless, an update
334 of this review would be useful because of the current standardized and optimized protocols
335 given in recent studies (27). Since December 2017, a study on the accuracy of HPV testing
336 (VALHUDES study) has been ongoing in order to compare the clinical sensitivity and the
337 specificity of the test on vaginal and urinary self-sampling with the HPV testing on cervical
338 samples. Five assays will be evaluated, including the Anyplex II HPV HR detection
339 (Seegene®). The VALHUDES protocol provides a framework for the validation of HPV
340 assays on self-sampling, potentially allowing the use of these assays in primary screening.

The reproducibility of this study in other countries would be interesting for generating additional comparative data (28).

In our study, invalid results were scarce despite the fact that many factors may impact HPV DNA amplification, such as the frequency of inhibitors in urine, storage of urine, centrifugation procedure, DNA extraction procedure, and HPV DNA assay (13). Nevertheless, only 0.7% invalid results were observed, suggesting that urine self-sampling at home is an efficient method to screen for HPV infection. The sensitivity of HPV detection increases in urine when urine samples were collected as first-void compared with random or midstream ($p = 0.004$) (14). For the CapU3 study, our sampling protocol recommended urine collection on the first-stream urine, but this, of course, could not be checked.

In conclusion, this study confirms better acceptance of urinary HPV testing for women who are reluctant to have a cervical smear. Urine self-sampling is a good alternative method to Pap smears because this well-known method is not invasive, is easy to do, and does not require specialized consultation. Urine self-sampling is effective and sensitive for detecting high-grade cervical lesions and provides an alternative to screening and following-up women reluctant to participate in the usual cervical screening program. Therefore, we conclude that urinary HPV testing may be relevant for women who do not have regular cervical smears and thus extend screening coverage in our department. Urine self-sampling could be used in the future as an approach to cervical cancer screening and may therefore become an important tool in cervical cancer elimination plans. This innovative approach is also the subject of many other research projects (27,28).

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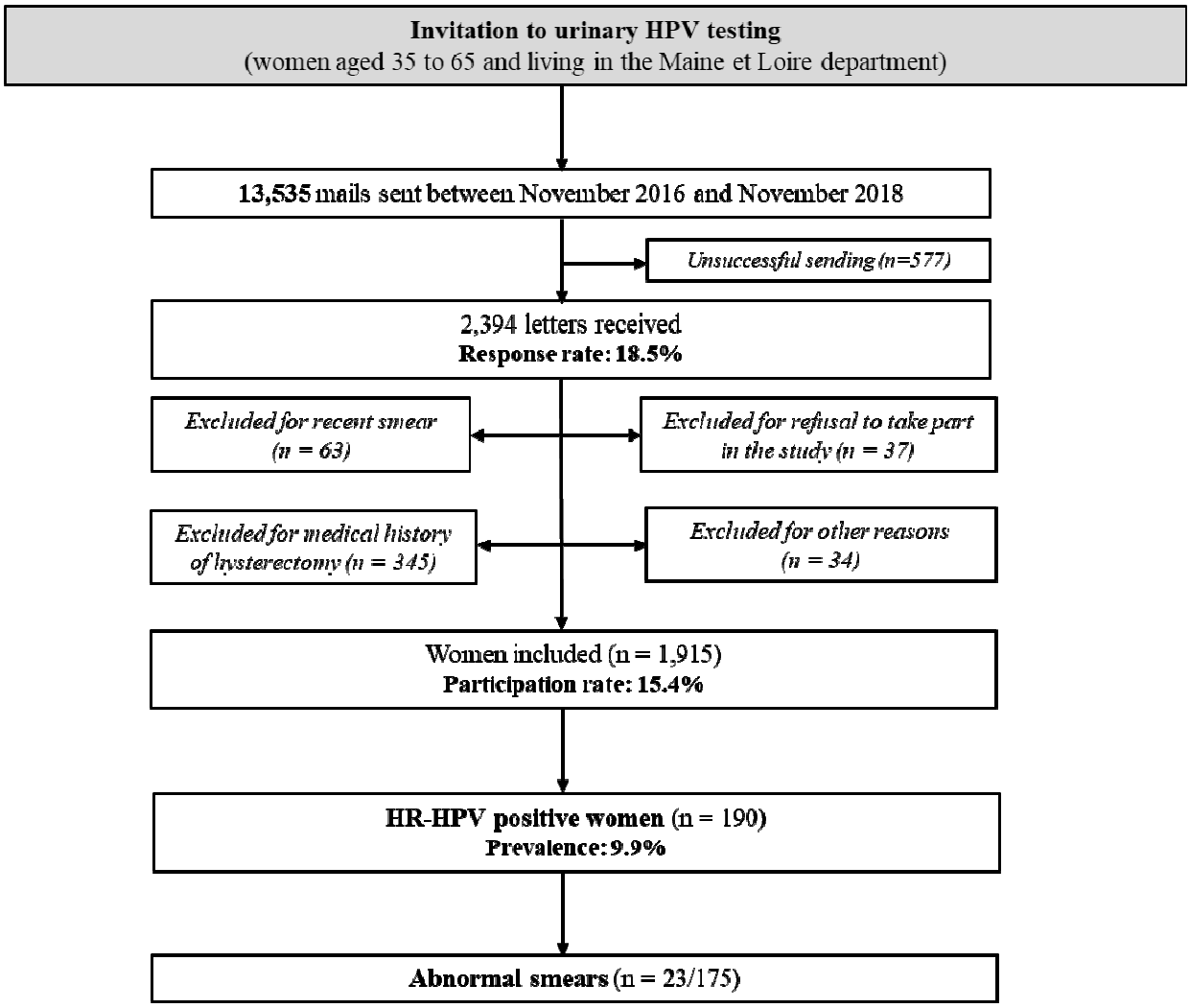

Figure 1: Flow chart describing response rate, participation rate, women excluded and included, rate of high-risk HPV-positive women and number of abnormal smears.

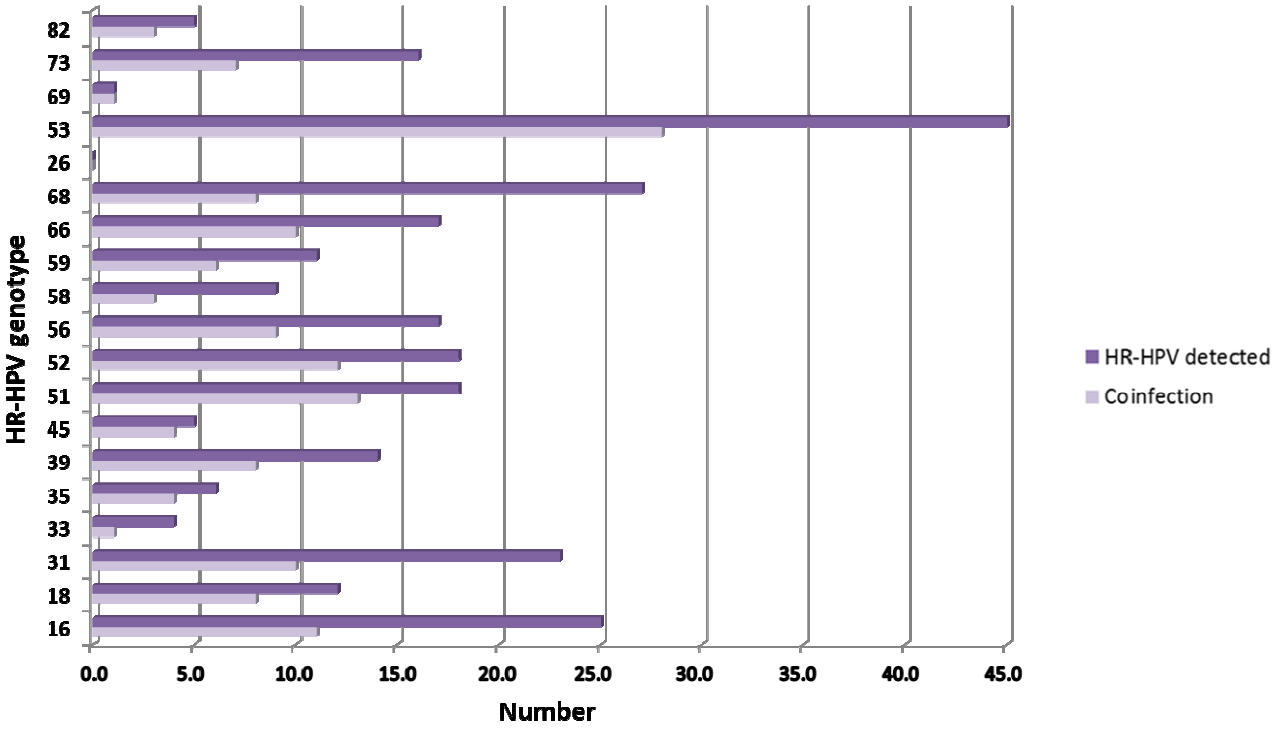

Figure 2: Distribution of HR-HPV genotypes among the 190 HR-HPV positive women. The dark purple bar corresponds to number of HR-HPV detected by types and the light purple bar represents number of HR-HPV detected by types in coinfection.

Table 1: The prevalence of HPV-16, HPV-18 and HR-HPV genotypes other than 16/18 in single infection or coinfection. 95% Confidence Interval (CI). Significant at $p<0.05$.

HPV groups	Prevalence	95% CI	*p* value*
HR-HPV genotypes other than 16/18	81.1% (154/190)	74.9-86.0%	
HPV-16 single infection	7.4% (14/190)	4.4-12.0%	p<0.0001
HPV-16 + HR-HPV coinfection	5.3% (10/190)	2.9-9.4%	p<0.0001
HPV-18 single infection	2.6% (5/190)	1.1-6.0%	p<0.0001
HPV-18 + HR-HPV coinfection	3.2% (6/190)	1.5-6.7%	p<0.0001
HPV-16 + HPV-18	0.5% (1/190)	0.09-2.9%	p<0.0001

* *p* value corresponds to comparison between prevalence of HR-HPV genotypes other than 16/18 and prevalence of each other group.

Table 2: Cytological and histological results of abnormal smears according to age and genotypes.

Age of patient	HPV Genotype	Cytological Result	Histological Result
41	51	HSIL	High-grade CIN 3
52	31, 53	HSIL	High-grade CIN 3
38	31	HSIL	High-grade CIN 3
43	16	HSIL	High-grade CIN 3
62	56	LSIL	High-grade CIN 3
61	16, 52	LSIL	High-grade CIN 3
46	33	LSIL	High-grade CIN 2
56	45, 53, 66, 73	LSIL	Low-grade CIN 1
64	52	LSIL	Low-grade CIN 1
50	82	LSIL	Low-grade CIN 1
60	39, 51	LSIL	Low-grade CIN 1
54	16, 51, 53, 66	LSIL	without dysplasia
58	51, 66	LSIL	without dysplasia
51	39	LSIL	without dysplasia
65	16, 39, 56	LSIL	without dysplasia
59	16	LSIL	without dysplasia
48	56	LSIL	without dysplasia
56	58	LSIL	*Missing data*
50	51, 66, 73	LSIL	*Missing data*
62	51	ASC-US	High-grade CIN 3
51	56, 66	ASC-US	Low-grade CIN 1
52	56	ASC-US	Low-grade CIN 1
62	58	ASC-US	without dysplasia

HSIL: high-grade squamous intraepithelial lesion; LSIL: low-grade squamous intraepithelial lesion; ASC-US: atypical squamous cells of unknown significance; CIN: cervical intraepithelial neoplasia.