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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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1 **Title:** Urinary HPV DNA testing as a tool for cervical cancer screening in women who are
2 reluctant to have a Pap smear in France.

3 **Running title:** Urinary HPV DNA testing for cervical cancer screening

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1 **Summary**

2 *Objectives:* In France, cervical cancer screening is based on human papillomavirus (HPV)
3 testing on cervical samples (women aged 30-65) and cytological examination of Pap smears
4 (25-29), but screening coverage is unsatisfactory. The CapU3 study aimed to propose urinary
5 HPV testing on 13,535 women aged 35 to 65 who had not had a Pap smear since 2010.

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

10 *Results:* The participation rate was 15.4%. Out of the 1,915 analyzed specimens, 1,711 and
11 190 were negative and positive, respectively, for at least 1 HR-HPV genotype. HR-HPV
12 genotypes other than HPV-16 or HPV-18 were mostly detected as HPV-53 (23.7%) and HPV-
13 68 (14.2%). A satisfactory gynecological follow-up was observed for HPV-positive women
14 (92.1%). 23 abnormal smears were observed and eight high-grade cytological lesions after
15 colposcopy and biopsy were diagnosed.

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

19

20

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

23

24 1

25 **Introduction**

26 In 2018, cervical cancer was the 4th most common cancer in women for incidence and
27 mortality after breast, colorectal, and lung cancer (1). In France, in 2017, it was the 12th most
28 common cancer with 2,800 new cases each year. Regarding mortality rate, it is 4th in the
29 world (about 270,000 deaths per year), 1st in low-income countries, and 12th in France (about
30 1,100 deaths per year) (2,3).

31 Cervical cancer screening over the last 50 years in Europe has significantly reduced the
32 incidence and mortality associated with cervical cancer. In France, between 1980 and 2019
33 (June), cervical screening was done through cytological examination of cervical-uterine
34 samples (Pap smear) in women aged 25 to 65 (4). However, at this time, despite the
35 campaigns and letters of encouragement to have a Pap smear, the screening coverage
36 remained low (below 60%) (2). **Vaccination coverage rate against HPV remained also low**
37 **(<30%) (5) in view of the objectives planned at 60% by the 2014-2019 Cancer Plan (6) and is**
38 **one of the lowest rates in Europe.**

39 Therefore, reflection tracks are being considered to improve screening coverage. High-risk
40 human papillomavirus (HR-HPV) testing on self-samples could be one way to increase access
41 to cervical cancer screening for women not participating in routine screening. Since July
42 2019, the French National Health Authority (*Haute Autorité de Santé*, HAS) has updated the
43 recommendations for cervical cancer screening and proposed a national screening strategy
44 that includes HPV testing. For women aged 30 to 65, cervical cancer screening is based on
45 HPV testing of cervical samples every five years. For women aged 25 to 29, cervical cancer
46 screening is based on cytological examination of a Pap smear every three years after two
47 consecutive annual smears (7).

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

65 In 2015, we reported the results of our CapU1 study, which offered urinary HPV testing to
66 5,000 women aged 45 to 65 years. We showed that urinary HPV testing may be pertinent to
67 women who had not had cervical Pap smears in three years. In doing so, the CapU1 study led
68 to the diagnosis of high-grade cervical lesions (three cases of cervical intraepithelial neoplasia
69 grade III lesions confirmed, CIN 3) (12).

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

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

77

78 **Material and Methods**

79 *Study design*

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

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

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

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

109 ***Response rate and participation rate***

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

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

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

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

125

126 ***Virological analysis***

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

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

138 The HPV DNA was then extracted from 1 mL of urine sample using the NucliSENS®
139 easyMAG® (Biomérieux). The HPV DNA amplification was performed according to the
140 manufacturer's instructions and 5 µL of nucleic acids were extracted from the urine sample.
141 The amplification of HPV DNA was performed on the CFX96™ thermocycler (Biorad®).
142 Internal control (IC) amplicons were used to assess the sample validity, extraction, and
143 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

176 **Results**

177 ***Response rate***

178 Between November 2016 and November 2018, 13,535 letters were sent to women aged 35-65
179 years. Of the 13,535 letters sent, 12,958 reached their respective addressees and 577 were
180 unsuccessful, i.e. 4.3% (not living at the stated address, referral, or damaged mailboxes).
181 2,394 letters with or without urine samples were received at our laboratory, i.e. 18.5% of
182 responses.

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

185 ***Participation rate***

186 Of these responses, 479 women were excluded from the study for the following reasons: 345
187 women for medical history of hysterectomy; 63 women due to recently having had a smear
188 test; 37 women refused to participate in the study; 34 women for other reasons, such as patient
189 residing outside the department, death, incomplete file, hospitalization, or unidentified reason
190 (Figure 1). 1,915 women were included in the CapU3 study (letter received with the urine
191 sample and signed consent without exclusion criteria), with a participation rate of 15.4%
192 (Figure 1). Indeed, the participation rate was significantly different between age groups 45-49
193 (16.9%) and 50-54 (14.2%) ($p = 0.014$); between age groups 45-49 and 55-59 (14.1%) ($p =$
194 0.020); between age groups 45-49 and over 60 years old (13.4%) ($p < 0.001$). The mean age
195 of women included was 53.8 ± 8.5 years.

196 ***HPV prevalence***

197 Out of the 1,915 samples analyzed, 1,711 were negative and 190 were positive (detection of
198 HR-HPV), i.e. an HR-HPV prevalence of 9.9% (95% Confidence Interval, [CI]: 8.7%-
9

199 11.3%). There were only 14 samples with no internal control amplification (0.7%) and thus
200 HPV DNA detection could not be validated. The mean age of HPV-infected women was 54.2
201 ± 8.7 years and the mean age of non-HPV infected women was 53.8 ± 8.5 years.

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

208

209 Independently of single infection- or coinfection, the distribution of HR-HPV types showed
210 that the main genotype identified was HPV-53 (23.7%) followed by HPV-68 (14.2%), HPV-
211 16 (13.2%), and HPV-31 (12.1%). HPV-18 was detected in only 6.3% of cases (Figure 2).

212 **Distribution of HR-HPV types among HR-HPV positive women and according to IARC**
213 **classification was: 131/190, 68.9% group 1; 21/190, 11.1% group 2A and 38/190, 20.0%**
214 **group 2B.**

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

217 **Gynecological follow up, cytological and histological results**

218 190 women were referred to their general practitioners or gynecologists for a cytology test
219 and/or colposcopy following a positive urinary HPV test. Of the 190 HPV-positive women,
220 175 of them carried out a Pap smear and one woman was monitored for anal cancer (in this
221 case, Pap smear is contraindicated for two years after radiotherapy). Therefore, the percentage
222 of Pap smears performed after a urinary HPV test was 92.1%. The cytological results showed

223 148 (84.6%) satisfactory Pap smears without dysplasia, 23 (13.1%) abnormal Pap smears, two
224 inflammatory Pap smears (1.1%), and two unsatisfactory Pap smears (1.1%). Of the 23
225 abnormal smears, four HSIL (17.4%), 15 LSIL (65.2%), and four ASC-US (17.4%) were
226 diagnosed. Colposcopy with biopsy confirmed six CIN 1 lesions, eight CIN 2-3, and seven
227 without dysplasia (Table 2). Seven CIN 3 lesions correspond to four HSIL, two LSIL, and one
228 ASC-US. The prevalence of high-grade lesions in the present study was 8/23 abnormal
229 smears, i.e. 35%. Despite reminders, two women refused to consult their gynecologists to
230 have a colposcopy.

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

235

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

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

243

244 **Discussion**

245 In this study, the main objective of increasing cervical cancer screening coverage in our
246 department was largely achieved as we invited almost all women aged 35 to 65 years, i.e.
247 13,535 women, who had not yet had a Pap smear since 2010.

248 The CapU3 study has shown high levels of acceptance of urinary self-sampling by
249 participants. We gained two participation points compared to the previous CapU1 study
250 conducted in our department. Indeed, in the CapU3 study, we estimated a participation rate of
251 15.4% versus 13.7% in the CapU1 study (12), while the women invited to the CapU3 study
252 had not had a Pap smear for at least seven years versus three years in the CapU1 study. The
253 CapU1 study was the first to evaluate a new strategy involving HPV detection in urinary self-
254 sampling at home, in addition to a cervical cancer screening program. In our study, it would
255 appear that the participation rate in the 45-49 age group was higher but this result must be
256 interpreted with caution because of a larger number of exclusions in the age group of 55 years
257 old and above (hysterectomy).

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

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

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

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

288 The CapU3 study confirms our previous results on the predominance of HR-HPV types other
289 than genotypes 16/18 among the HPV cases detected, regardless of the cytological results
290 (12). It was primarily the HPV-53 genotype detected in our target population with detection in
291 23.7% of HPV-positive samples followed by genotype 68 detected in 14.2%. HPV-16 and

292 HPV-18 represented 13.2% and 6.3%, respectively, of HPV-positive samples, regardless of
293 the cytological results and these results were equivalent to the prevalence described in France:
294 15.2% for HPV-16 and 2.4% for HPV-18 (24).

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

297

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

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

313 In this study, we reported a predominance of HR-HPV types other than HPV-16/HPV-18 in
314 abnormal smears. In the French HPV reference laboratory, HPV prevalence increased with
315 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 marketed 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.

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

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

351

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

363

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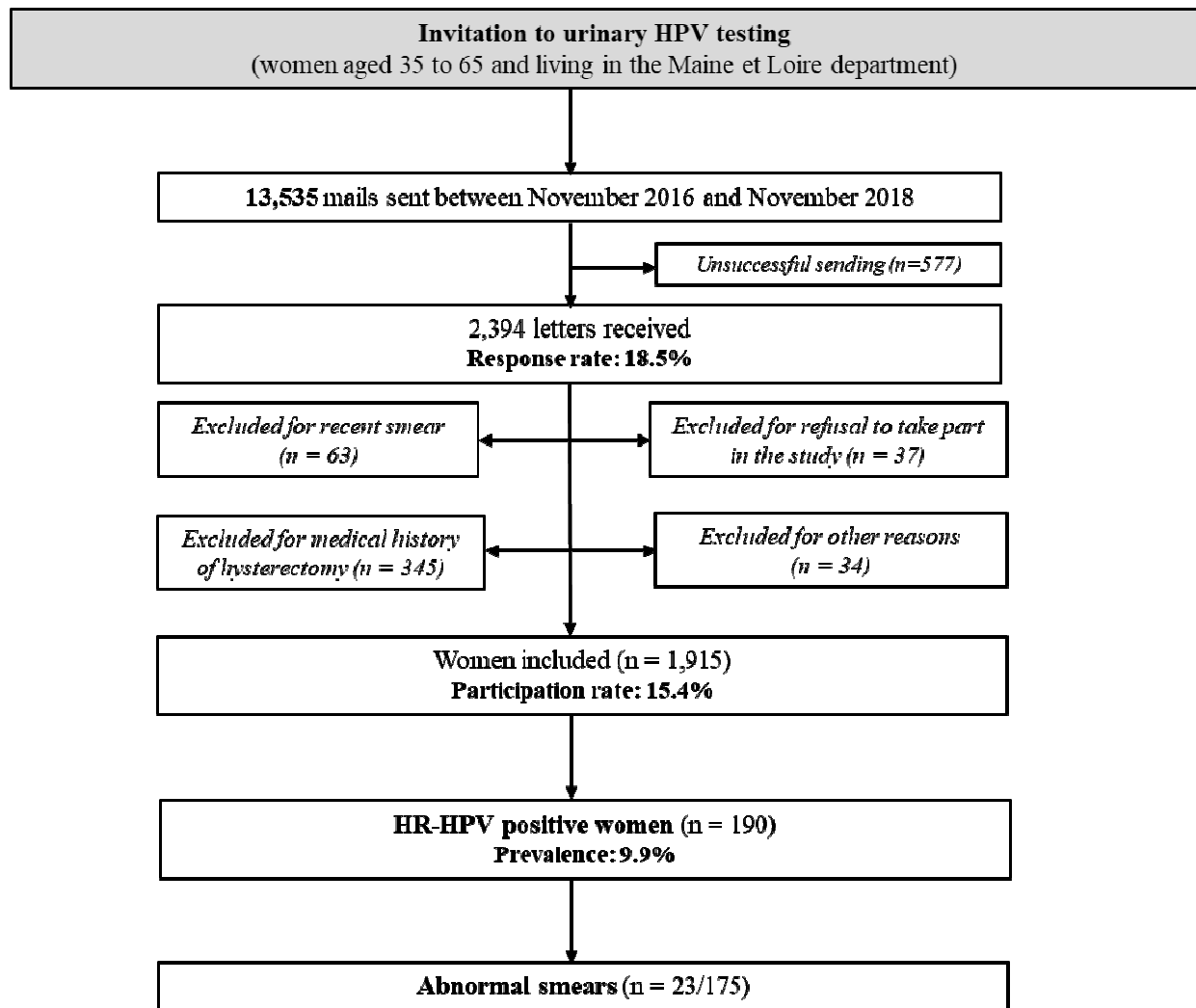


Figure 1: Flow chart describing response rate, participation rate, women excluded and included, rate of high-risk HPV-positive women and number of abnormal smears.

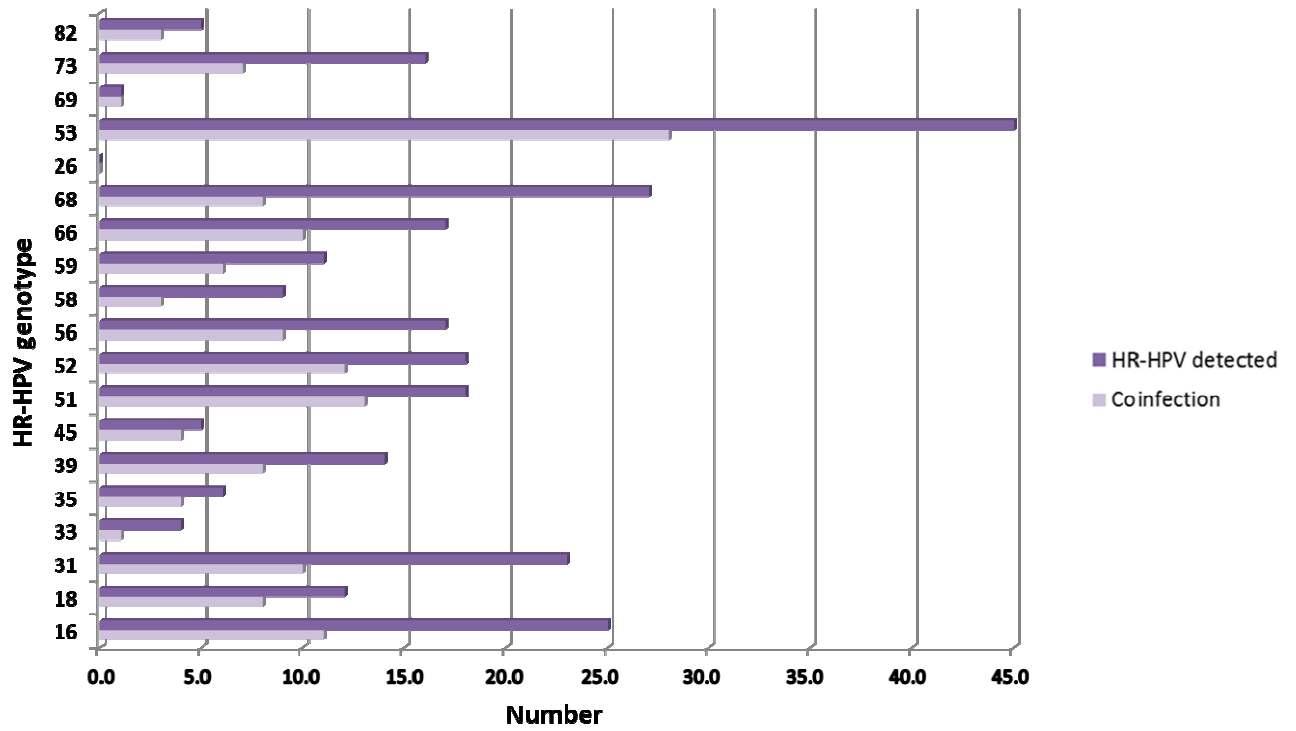


Figure 2: Distribution of HR-HPV genotypes among the 190 HR-HPV positive women. The dark purple bar corresponds to number of HR-HPV detected by types and the light purple bar represents number of HR-HPV detected by types in coinfection.

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	<i>p</i> 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.