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Follow-up of humoral immune response after HPV vaccination using first-void urine: a longitudinal cohort study

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Abstract

Assessment of humoral immune responses following human papillomavirus (HPV) vaccination currently relies on invasive blood sampling. This longitudinal cohort study explores the usability of first-void urine as a non-invasive alternative sample for antibody detection. In this study, 58 women receiving three doses of the 9vHPV vaccine within a Gardasil9 (9vHPV) Phase III randomized controlled trial were included. Participants provided paired first-void urine and blood samples before vaccination (M0), one month after the third dose (M7), and approximately three years after the third dose (M43). Type-specific antibody responses to the 9vHPV types were analyzed in 174 first-void urine and 172 serum samples using a virus-like particle (VLP)-based IgG multiplex ELISA. Additionally, total IgG concentrations were determined using the BioPlex assay. At M7, one month after complete 9vHPV vaccination, 95–100% of first-void

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Ethics statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of UZA/ University of Antwerp (B300201734258, 27 November 2017). Informed consent was obtained from all subjects involved in the study.

Other authors declare that they have no conflict of interest.

urine and 100% of serum samples had detectable concentrations, varying by HPV type. At M43, 84–100% of first-void urine and 98–100% of serum samples had HPV-specific antibody concentrations. Results show significant Spearman rank correlations between type-specific HPV-antibody concentrations for paired first-void urine and serum at all time points. This study confirms the potential feasibility of utilizing first-void urine as a non-invasive immunological sample within HPV vaccine trials.

Keywords

First-void urine; Human papillomavirus; HPV; Vaccination; Antibody; Humoral immunity

Introduction

The possibility of using a single non-invasive, home-collected sample to monitor human papillomavirus (HPV) infection and vaccination is an interesting prospect. This study focuses on investigating the potential of the initial stream of urine, defined as first-void urine, as a means to achieve this objective ¹. A first-void urine sample contains accumulated genital secretions between the labia minora and urethra opening. These secretions can include HPV DNA, transudated antibodies, methylation markers, and other HPV-related biomarkers (Figure 1) ^{2–6}. Our research group at the University of Antwerp, Belgium, recently confirmed that transudated HPV vaccine-induced antibodies are detectable in the first-void urine of young women. In this study, significantly higher HPV-specific antibody levels were found in the first-void urine of vaccinated compared with unvaccinated women. Moreover, positive correlations were observed between antibodies to the HPV types included in the quadrivalent HPV vaccine (4vHPV) in first-void urine and paired sera using two different immunoassays not optimized for first-void urine samples ^{7,8}.

Cervical cancer can be prevented most effectively by combining HPV vaccination with cervical screening. In the absence of a correlate of protection, it is generally accepted that the HPV-type specific antibodies produced after vaccination or natural infection are indicators of protection against HPV infection ^{9–12}. Screening and immunomonitoring during HPV vaccine trials have relied mainly on serology and cervical samples ¹³. First-void urine could be ethically acceptable to determine the pre-vaccination HPV DNA status in girls too young to be exposed to a gynecological examination or to take a vaginal self-sample, as already demonstrated in the HPV vaccine monitoring programs in Bhutan and Rwanda ^{14–17}. Furthermore, non-invasive, easily obtained, at-home sampling can simplify the operational management and logistics of HPV vaccine trials, therefore potentially leading to fewer lost-to-follow-up, lower trial costs, and increased sampling frequency if needed ¹⁸.

Since limited data are available on the evaluation of first-void urine as an alternative sample for HPV antibody detection, this longitudinal cohort study, nested in a randomized controlled vaccine trial (RCT), was set up. This study is the first to assess if first-void urine is a suitable tool for monitoring HPV vaccination with the nonavalent HPV vaccine (9vHPV) in women, in combination with HPV DNA detection.

Methods

Study design and participants

This longitudinal cohort study was nested in a phase III randomized controlled trial in which volunteers were vaccinated with 9vHPV (Merck & Co., Inc) at 0, 1, and 6 months (HPV V503–004 study; EudraCT NUMBER: 2015–005093–38) ¹⁹. Recruitment occurred in Belgium at the Centre for the Evaluation of Vaccination (CEV), University of Antwerp (UA) and Antwerp University Hospital (UZA) from December 2017 to February 2018 (visit 1, M0), with additional visits in June–October 2018 (visit 2, M7), and August–September 2021 (visit 3, M43) (Figure 2). Key exclusion criteria included incomplete 9vHPV vaccination (three doses), cervical cancer or precancerous treatment in the last six months, and participation in any other interventional clinical trial.

In this sub-study, women participating in the phase III clinical trial (HPV V503–004) were asked if they were willing to provide first-void urine samples in addition to blood samples at M0 and M7. Women willing to participate were sent the information brochure, informed consent form, and practical instructions by e-mail. They were asked to read these documents thoroughly before participating in the study. Approximately three and a half years after initial inclusion, women were contacted again and asked if they were willing to provide another first-void urine sample and a blood sample (M43). All volunteers were asked to complete a questionnaire at each visit, including information on the menstruation cycle and contraception. At M43, additional information on demographics and their sexual status were collected. Informed consent was obtained from all volunteers and data was coded to ensure privacy of the participants. The institutional review board of the Biobank and the ethical committee of Antwerp University Hospital (UZA)/UAntwerp, Belgium (B300201734258) approved all study procedures, and the study is registered with [clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT03542227) (NCT03542227).

Sample collection

Before each first-void urine sample collection, we asked the women to not wash their genitals thoroughly, not to use a tampon, and not to urinate at least two hours before collection. All first-void urine samples were collected using the Colli-Pee[®] 20 ml device (Novosanis, Wijnegem, Belgium) prefilled with a urine conservation medium (UCM). Samples were collected at the CEV or UZA refrigerated for up to 4 hours, vortexed, aliquoted, and stored at –80°C (Biobank Antwerpen, Antwerp, Belgium; ID: BE 71030031000) before further analysis ²⁰. Before aliquoting, the presence of erythrocytes in first-void urine was determined using Hemastix[®] reagent strips for urinalysis (Siemens Healthcare Diagnostics Inc., Dilbeek, Belgium). Blood samples were collected at M0 and M7 as part of the 9vHPV phase III clinical trial ¹⁹, and at M43 as part of the sub-study. We obtained 200 µl aliquots of serum from the M0 and M7 samples from Merck & Co, Inc. These aliquots were stored at –80°C until further use. For the M43 samples, blood was collected using 10 ml BD Vacutainer[®] Serum Tubes without anticoagulant (BD Benelux N.V, Erembodegem, Belgium) and allowed to clot for 30–60 minutes. The samples were then centrifuged at 1000 g for 10 minutes at 20 °C, and the serum was divided into aliquots before being stored at –80 °C.

HPV DNA testing

HPV DNA testing was performed on first-void urine samples. Buffered aliquots were thawed before sample preparation. HPV DNA testing for HPV16, HPV18, and 12 other High-Risk (HR)-HPV types (HPV31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) was performed at the Centre for Medical Analysis (CMA, Herentals, Belgium) on 1 ml of buffered first-void urine using Cobas 6800 (Roche Molecular System, Pleasanton, CA, USA) and the clinical cut-off for cervical samples. If positive on Cobas 6800, we genotyped the sample. For this, DNA was extracted using an in-house protocol²¹. Briefly, 4 ml of first-void urine was centrifuged at 3,820 g for 20 min at 20°C in an Amicon Ultra-4 50K filter device (Merck Millipore, Brussels, Belgium). Next, 2 ml NucliSENS Lysis Buffer (bioMérieux Benelux, Schaerbeek, Belgium) was added to the concentrate retained on the filter. After incubation at room temperature, all material was transferred to the NucliSENS Lysis buffer vial for DNA extraction. DNA was eluted in 55 µl, and 35 µl was transferred to a vial with ultrapure water, resulting in a total volume of 75 µl DNA extract for HPV DNA genotyping. HR-HPV DNA-positive samples were genotyped using the Riatol qPCR HPV genotyping assay. This assay quantifies beta-globin and 18 HPV genotypes (HPV6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 67, 68) as described by Depuydt et al.²².

HPV-specific antibody testing

For antibody testing, first-void urine samples were concentrated. Thawed 4 ml aliquots were centrifuged at 3,820 g for 10 min at 20°C using an Amicon Ultra-4 50K filter device (Merck Millipore, Brussels, Belgium). 1X dPBS (Dulbecco's phosphate-buffered saline, Gibco, Painsley, Scotland, UK) was added to the concentrate retained on the filter to reach a final volume of 0.5 ml. First-void urine concentrates were stored at -80°C until further testing.

HPV type-specific antibodies were quantified using the HPV 9-plex VLP-based IgG ELISA (M9ELISA) as described by Panicker et al. (2021)²³. First-void urine samples were tested starting undiluted, whereas serum samples (including standards and controls) testing started at 1:100 dilution or higher to ensure that the generated signal was within the linear range of the assay. A minimum of three dilutions were tested for each sample. The plates were read on the MESO® Quickplex SQ120 (Meso Scale Diagnostics LLC, Rockville, MD, USA). HPV antibody concentrations were determined using raw relative light units (RLUs) for serum samples and background subtracted RLUs (signal from bovine serum albumin (BSA) spot subtracted) for concentrated first-void urine samples using the parallel line method (PLL) described in the World Health Organization (WHO) HPV Labnet Manual 2009^{24,25}. A serum or first-void urine sample passed PLL conditions if (1) the correlation between the three selected dilutions was ≥ 0.9 , (2) the absolute value of the slope was ≥ 0.4 , (3) the ratio of the slope of the standard and the test sample was ≥ 0.5 , and (4) if there was not more than one data point out of three points outside the linear range. We report antibody concentrations in arbitrary units/ml (AU/ml) for all types except HPV16 and 18, which are reported in International Units (IU/ml). The HPV16 (05–134) and HPV18 IU standards (10–140) were obtained from the National Institute for Biological Standards and Controls [NIBSC], Potter's Bar, UK. A serum sample was given a concentration if it passed all PLL conditions and if the concentration was at or above the lower limit of quantification (LLOQ,

only defined for serum). The LLOQ values for serum are as 0.2 AU/ml for HPV6, 0.4 AU/ml for HPV11, 1.0 IU/ml for HPV16, 0.3 IU/ml for HPV18, 1.6 AU/ml for HPV31, 3.0 AU/ml for HPV33, 2.3 AU/ml for HPV45, 1.6 AU/ml for HPV52, and 2.2 AU/ml for HPV58. A first-void urine sample was given a concentration if it titrated linearly and passed all PLL conditions, as no LLOQs were defined yet.

Total human IgG testing

Total human IgG concentrations were determined using the BioPlex Pro™ Human Isotyping Assay for total human IgG (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. We diluted concentrated first-void urine samples at 1:128 and 1:512 and serum samples at 1:10,000 and 1:100,000. Measurements were performed using the LX200 platform (Luminex, Austin, Texas, USA). The software calculated concentrations from the median fluorescence intensity (MFI) values using a five-parameter logistic regression. Total human IgG concentrations were calculated as the average of two dilution-corrected concentrations.

Statistical analysis

We performed statistical analysis using R statistical software version 4.2.2 (packages: rstatix, tidyverse, irr, RVAideMemoire). If the data were normally distributed (Shapiro–Wilk test and Q-Q plot), significant differences in mean antibody concentrations between the three visits were examined using a one-way repeated measures ANOVA. Otherwise, a non-parametric Friedman test was used. This was followed by a post hoc paired t-test (parametric) or Wilcoxon signed rank test (non-parametric) for multiple paired comparisons. We used the Spearman rank test to calculate the correlation between antibody levels in different samples and bootstrap 95% confidence intervals (CI) were calculated using 1,000 reps. Cohen's kappas (κ) and the corresponding 95% CI were calculated to assess the antibody agreement between sample types (κ 0.20, poor; 0.21 κ 0.40, fair; 0.41 κ 0.60, moderate; 0.61 κ 0.80, good; and κ 0.81, excellent agreement). For analyses, statistical significance was defined as p-adjusted < 0.05 (using Holm–Bonferroni method for p-value adjustment). We performed all analyses on untransformed data. Figures were made using antibody concentrations or $\log_{10}(x)$ transformed data where zero values were assigned as 0.0001.

Results

Population characteristics

Between Dec 11, 2017, and Oct 12, 2018, 63 out of 81 contacted women participating in the phase III randomized controlled trial consented to be enrolled in this sub-study (Figure 2). From Aug 26, 2021, to Sep 30, 2021, all 63 women were contacted again, and 58 participants were reached. We obtained first-void urine at all time points from all 58 included women. From one woman, we did not receive consent to test serum samples collected at M0 and M7 (Figure 2). Median time between the M0 and M7 visit was 6.4 months (IQR 6.2–7.1), and the median time between the M0 and M43 visit was 43.4 months (IQR 42.7–44.0). Median age at enrollment was 35 years (IQR 27–41). Of all included women, 97% (56/58) had been sexually active before enrollment, with a median age at first

sexual encounter of 17 years (IQR 16–19). The median number of sexual partners was 4 (IQR 4–6). 86% of the women had provided a cervical sample in the past (50/58), and the median age of providing the first cervical sample was 20 years (IQR 18–25). 22% of the women (13/58) had a smoking history, and 69% (40/58) of the women had given birth at least once. The most common contraceptive used was an intrauterine device, 47% (27/58), and 4% (2/58) used a condom (Table 1).

HPV DNA results

Using the Cobas 6800 assay, we identified 8/58 (14%) women with an HR-HPV infection in first-void urine before vaccination. At M7, 14% (8/58) of the women had an HR-HPV infection, two M0 infections cleared, and two new infections were detected. At M43, 7% (4/58) of the women tested HPV DNA positive, six M7 infections were cleared, and two new infections were detected. All samples that were HPV DNA positive on the Cobas 6800 assay were genotyped using the Riatol qPCR. A total of five women had infections with HPV types included in the 9vHPV vaccine (HPV16,31,33,52,58). One woman had HPV16 and HPV33 DNA detected at all visits, and one had detectable HPV58 DNA at M43 (Table 2).

Detection of HPV-specific antibodies

HPV-specific antibody detection and median antibody concentrations were determined for first-void urine and serum samples (Table 3). Before vaccination, we detected HPV-specific antibodies in 0% (0/58, HPV52) to 16% (9/58, HPV6) of the first-void urine samples. One month after the third 9vHPV vaccine dose, 95% (55/58, HPV45) to 100% (58/58, HPV11–16–31–33–58) of first-void urine samples had detectable antibody concentrations, and 84% (49/58, HPV52) to 100% (58/58, HPV16) at M43. In serum, we detected HPV-specific antibodies in 9% (5/57, HPV33) to 37% (21/57, HPV6–18) samples collected before vaccination. One month after the third dose of the 9vHPV vaccine, antibody detection in serum was 100% (57/57) for all HPV types, and 98% (57/58, HPV52) to 100% (57/57, all other HPV types) at M43. For all samples, there were significant differences in HPV-specific antibody concentrations between all different collection points for each HPV type (Figure 3). HPV-specific antibody concentrations were nearly 1,700 times higher in serum than first-void urine for all samples at each time point and were about 10-fold lower for all samples at M43 than one month after complete vaccination (M7). Total IgG antibody concentrations in serum samples were approximately 200-fold higher compared to first-void urine. Significant positive Spearman rank correlations were found between serum and first-void urine HPV-specific antibody concentrations. Correlations coefficients ranged between 0.36 (95% CI 0.002–0.64) (HPV18) and 0.79 (95% CI 0.46–1.00) (HPV33) for M0, between 0.54 (95% CI 0.30–0.75) (HPV11) and 0.71 (95% CI 0.51–0.83) (HPV45) for M7, and between 0.64 (95% CI 0.41–0.79) (HPV11) and 0.82 (95% CI 0.71–0.89) (HPV33) for M43 (Table 3) (Appendix Figure 1)²⁶. The antibody detection agreement (k) between serum and first-void urine was moderate to excellent and ranged between 0.68 (95% CI 0.56–0.81) (HPV6) and 0.93 (95% CI 0.87–0.99) (HPV16 and HPV33) (Table 3).

Finally, we combined the data on HPV detection and antibody concentrations for the women with HPV DNA detected at least at one of the visits (Appendix Figure 2). Only four women

had HPV DNA from HPV types included in the 9vHPV vaccine in their first-void urine sample. For two of these women, we detected a naturally induced HPV-specific antibody response in the first-void urine samples to the specific type before vaccination. In addition, we provide an overview of elicited antibody concentrations for all samples at all visits (Appendix Figure 2).

Discussion

In this longitudinal cohort study, we provide the first data supporting the potential of first-void urine to monitor HPV-specific antibody response in a vaccine trial. The data show that we can detect HPV-specific antibodies to all 9vHPV vaccine types at peak levels and that they remain detectable in first-void urine for at least three years after vaccination. We were also able to measure HPV DNA status and detect naturally induced immunity using this sample type. Antibody concentrations after vaccination in first-void urine correlated well with serum concentrations for all HPV types at M7 and M43. In addition, we found significant correlations between serum and first-void urine antibody levels before vaccination (M0). As serum antibody levels are a good measure of protection in the absence of a correlate of protection, being able to measure the antibody levels at the site of infection could be helpful in the search for a correlate of protection against HPV infections and associated diseases.

With first-void urine, we collect the female genital secretions from, e.g., the uterus, cervix, and vagina that accumulated between the labia minora. Since the main Ig class in the genital tract is IgG, it was expected that these antibodies would also be abundant in the genital secretions captured with first-void urine^{27,28}.

While antibodies were detected in most first-void urine samples at both M7 (>95%) and M43 (>84%), some samples had undetectable concentrations using the current M9ELISA. Since the assay was developed for serum, using a concentrated first-void urine sample could affect the background and sensitivity of the assay specifically optimized for diluted serum samples. For the first-void urine samples, HPV-specific antibody concentrations were calculated after subtracting the signal in the control spot (BSA) to mitigate the impact of non-specificity arising from the concentrated sample. The laboratory protocol for serum M9ELISA does not use background subtraction in concentration calculations, as backgrounds are generally low. However, occasionally samples do show high backgrounds. In the current study, two serum samples had high values in the BSA control spot, suggesting potential non-specificity. The effect of BSA will be further evaluated during assay optimization. Undetectable HPV-specific antibodies in first-void urine compared to serum could also be due to incorrect sampling. As first-void urine samples were collected at the clinical trials center instead of at home, women might be more tempted to wash their genitals, resulting in fewer secretions being captured in the first void of urine.

Most of the HPV infections detected in the first-void urine samples were from HPV types not included in the 9vHPV vaccine. Looking at the limited combined data of the HPV DNA detection and HPV-specific antibody response, we observed naturally induced antibody responses before vaccination. The latter was expected in this sexually active cohort as

serology is a marker of past or present infection. As first-void urine has detectable HPV-specific antibody concentrations after natural infection, detecting antibodies after one-dose HPV vaccination might be feasible since these concentrations are known to be two to four times higher than after natural infection ²⁹.

The use of first-void urine sampling for primary and secondary cervical cancer prevention offers a number of advantages such as non-invasiveness, not requiring trained personnel, ease of collection outside a clinical setting, and reducing lost-to-follow-up and cost for vaccine and epidemiological trials. This approach is particularly important for monitoring the vaccine effectiveness and efficacy of current vaccination programs and one-dose HPV vaccination ^{29,30}, reducing issues such as lost-to-follow-up and increasing compliance ^{26,31}. This alternative method could also be used to evaluate new prophylactic or therapeutic HPV vaccines ³². Furthermore, first-void urine sampling could be suitable for detecting other sexually transmitted infections, e.g., Chlamydia trachomatis, and for the evaluation of corresponding vaccines under development ³³.

This pilot study has certain limitations. Due to the absence of existing data on HPV-specific antibody detection in first-void urine, a formal sample size calculation was not feasible. Nevertheless, our longitudinal analysis involved a total of 346 samples (174 first-void urine and 172 serum samples) and results for nine different HPV types. This sample size provides robust results to adequately describe the potential of a first-void urine sample for vaccination follow-up. We only included female participants in this study since first-void urine sampling only applies to women. Since the amount and origin of genital tract secretions differ between males and females, the secretions captured with first-void urine are also different. Optimizing the existing assays and further concentrating the urine samples may improve the detection of HPV-type specific antibodies in first-void urine. In addition, normalization for IgG fluctuations over the menstrual cycle ³⁴, and hormonal influence on the accumulation of vaginal mucus are being investigated. As first-void urine data using total IgG normalization did not provide better results compared to non-normalized data, we utilized non-normalized data in this manuscript. Nevertheless, we could still provide robust results even without assay optimization or normalization of the first-void urine data.

In conclusion, we are the first to demonstrate that first-void urine can be used in HPV vaccine trials to monitor HPV infection and immune responses, thereby replacing a clinician-taken cervical sample or vaginal self-sample and a blood sample by a non-invasive self-collected sample. Once validated, it can be a suitable sample for surveillance and follow-up of vaccine trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Conflict of interest statement:

A.V. is a co-founder and former board member of Novosanis (Subsidiary of OraSure Technologies Inc, Wijnegem, Belgium), a spin-off company of the University of Antwerp, and was a minority shareholder until January 2019. The University of Antwerp received grants from Merck, GSK, Hologic, Abbott, Roche, and Cepheid to support the HPV Prevention and Control Board. The University of Antwerp received a project grant and honoraria fee for lectures, presentations, and speaker bureaus from Merck.

Role of the funding source:

The study funders had no role in the study design, data collection, data analysis, data interpretation, or writing of the article. The authors had full access to all data in the study and had final responsibility for the decision to submit for publication.

Data availability statement:

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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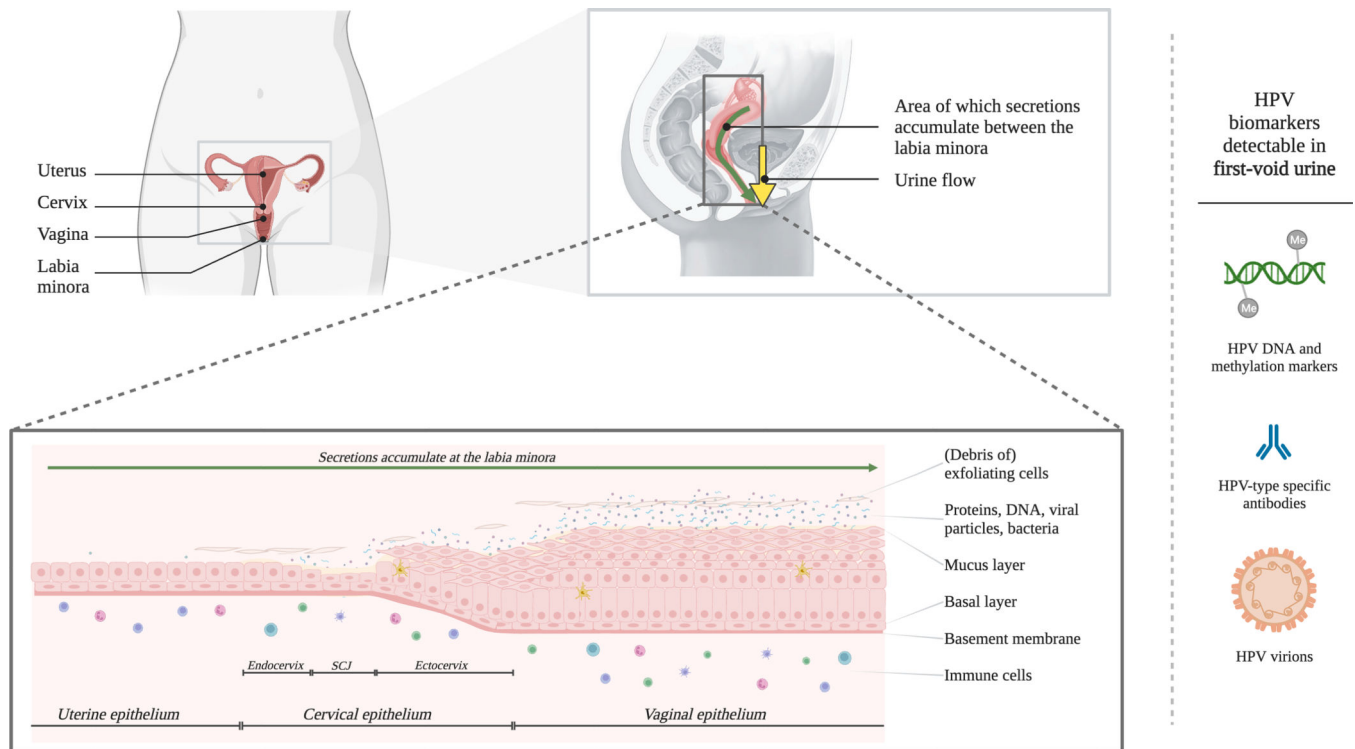


Figure 1: Overview of female genital secretions captured in first-void urine.

Secretions from the uterine, cervical, and vaginal epithelium transfer to the labia minora, where they accumulate. When the woman urinates, accumulated secretions are captured in the first fraction of the urine (e.g., first-void urine). Various proteins, DNA, viral particles, and bacteria are present in the vaginal epithelium, less in the cervical epithelium, and to the lowest degree in the uterine epithelium. SCJ= squamocolumnar junction.

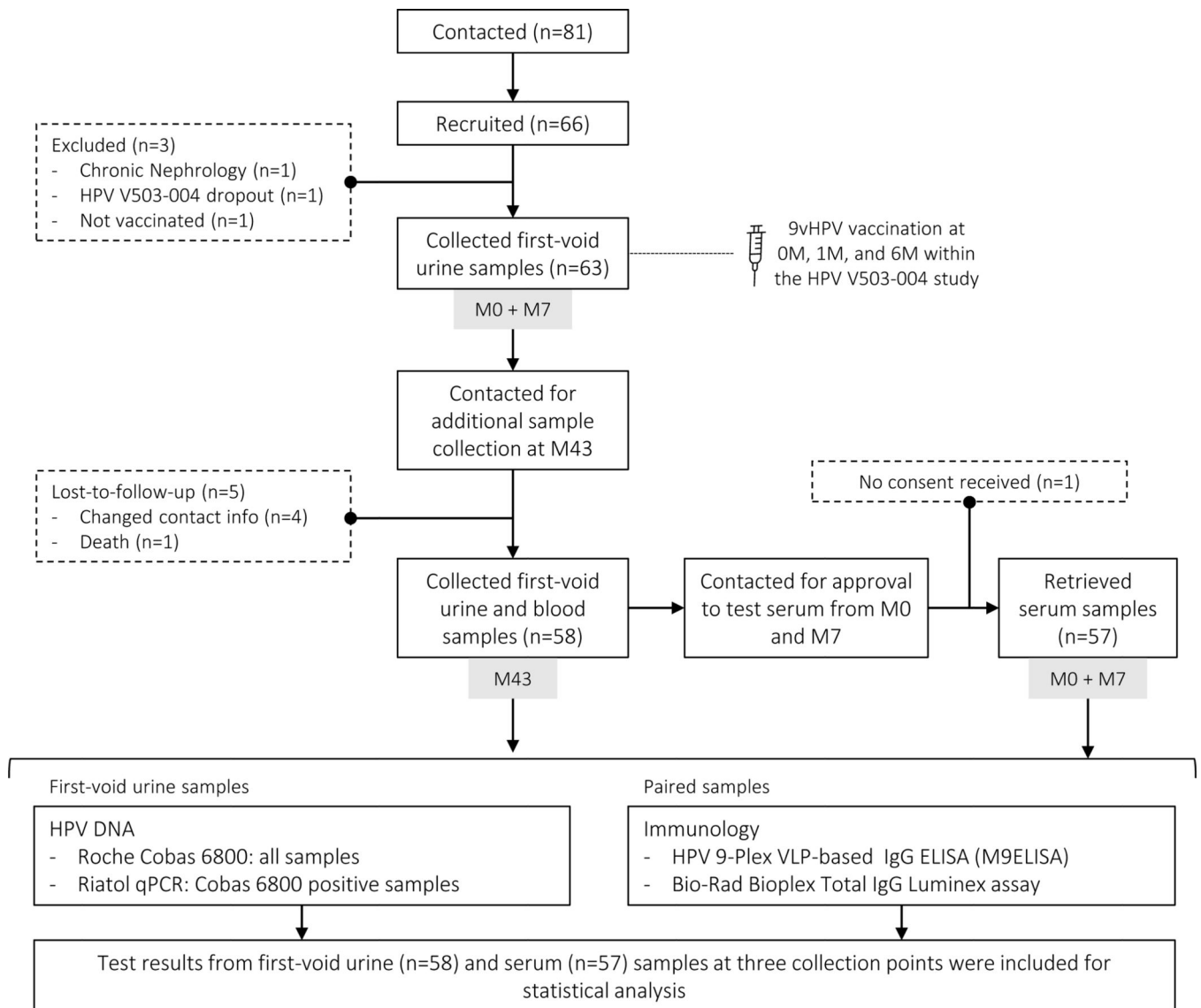


Figure 2: Study flowchart.

Sixty-six women were willing to provide first-void urine samples at M0 and M7, of which 63 eligible women were included. All 63 women collected first-void urine samples before vaccination (M0) and one month after the third 9vHPV vaccine dose (M7). We contacted all 63 women 3.5 years after the initial inclusion date to invite them for a follow-up visit. Fifty-eight women were reached and provided paired first-void urine and blood samples approximately three years after complete vaccination (M43). In addition, we contacted the women again to ask for their consent to test the serum samples collected at M0 and M7 as part of the HPV V503–004 study. The presence of HPV DNA was determined for each first-void urine sample using Roche Cobas 6800, and the HPV DNA-positive samples were genotyped using the Riatol qPCR HPV genotyping assay. All samples were analyzed for HPV-type specific antibodies (M9ELISA) and total IgG (BioRad). Data from 58 first-void urine and 57 serum samples were included for analysis.

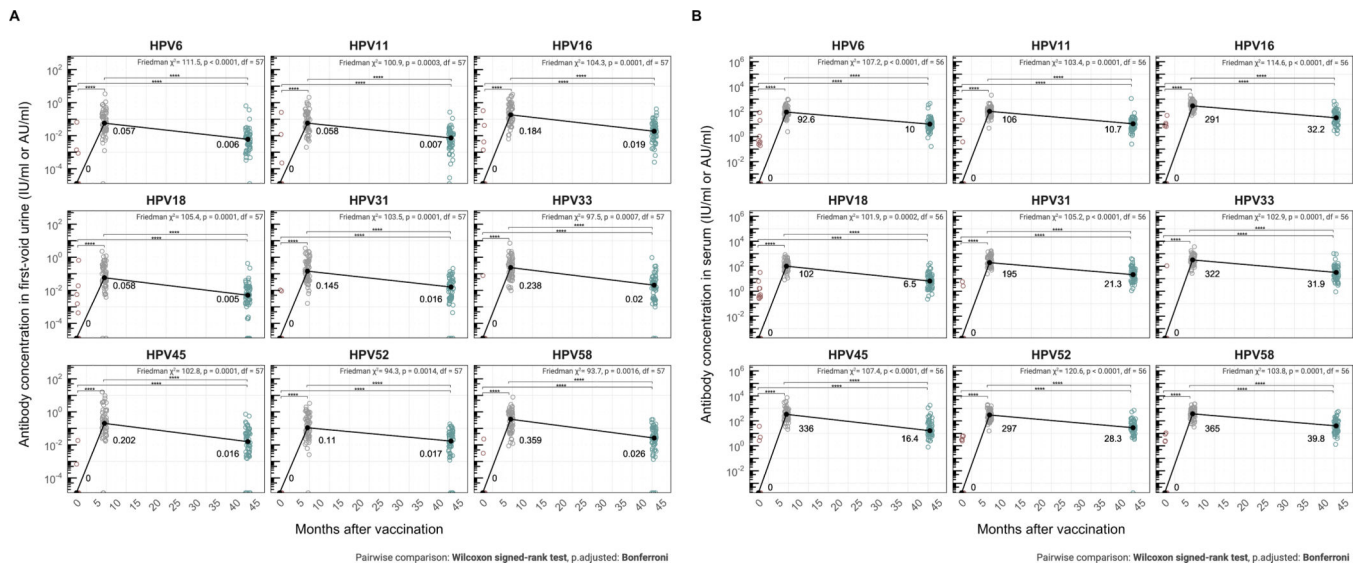


Figure 3: HPV-specific antibody concentration at M0 (pink), M7 (grey), and M43 (blue) measured in first-void urine samples (A) and serum samples (B). The median antibody concentrations are shown in the figure. Concentrations are expressed as IU/ml for HPV16 and HPV18 and as AU/ml for all other HPV types. A Friedman test was used for repeated measures analysis, followed by a Wilcoxon signed-rank test to analyze pairwise significant differences. Significant differences were found between all different time points for all HPV types and all sample types (****p-adjusted < 0.0001). AU=Arbitrary Units. IU=International Units.

Table 1:

Population characteristics and demographic data.

Characteristic	Number
Total	58 (100%)
Age, years	35 (27–41)
Time after previous urination, minutes	120 (90–165)
Sexually active	56 (97%)
Age at first sexual encounter, years	17 (16–19)
Number of sexual partners	4 (2–6)
Smoker	13 (22%)
History of cervical sample	50 (86%)
Age at first cervical sample collection, years	20 (18–25)
Already given birth	40 (69%)
Oral contraceptives	13 (22%)
Intrauterine device	27 (47%)
No contraceptives	16 (28%)
Condom	2 (4%)

Data are n (%) and median (IQR). Two women never had a sexual encounter, one volunteer did not provide details on the number of sexual partners, six volunteers did not know whether they had a cervical sample, eight volunteers did not know when they got the first cervical sample or did not provide a cervical sample yet, one volunteer used both oral contraceptives and condom at M7.

Table 2:

HPV DNA results in first-void urine samples.

ID	M0	M7	M43
	Riatol qPCR	Riatol qPCR	Riatol qPCR
14	HPV59	HPV59	HPV58, HPV59
19	None	None	/
24	/	HPV66	/
28	/	HPV52	/
31	HPV33, HPV59	/	/
35	HPV51	HPV51	/
37	HPV56	HPV56	/
45	/	/	HPV51
48	/	/	HPV67
51	HPV16, HPV33, HPV51	HPV16, HPV33, HPV51, HPV56, HPV66	HPV16, HPV33
60	HPV31, HPV51	HPV35, HPV51	/
62	HPV51	/	/

HPV types of which DNA was detected using the Riatol qPCR is presented here. Only samples that had a positive result for HR-HPV DNA other, HPV16, and/or HPV18 by Roche Cobas 6800 were tested using Riatol qPCR. One ID (ID19) had a positive result for HR-HPV DNA other using the Roche Cobas 6800 at M0 and M7 but was negative for the Riatol qPCR.

Table 3:

Summary of antibody detection and concentrations after 9vHPV vaccination.

Type	Months	First-void urine		Serum		First-void urine vs Serum concentration		First-void urine vs Serum detection
		Antibody detection	Antibody concentration (AU-IU/ml or µg/ml)	Antibody detection	Antibody concentration (AU-IU/ml or µg/ml)	Spearman Correlation (95% CI)	Adjusted p-value	Cohen's Kappa (95% CI)
HPV6	0	9/58 (16%)	0.000 (0.000–0.000)	21/57 (37%)	0.000 (0.000–0.380)	0.44 (0.12–0.68)	< 0.0001	
	7	57/58 (98%)	0.057 (0.022–0.203)	57/57 (100%)	92.60 (56.30–137.00)	0.63 (0.42–0.79)	< 0.0001	0.68 (0.56–0.81)
	43	57/58 (98%)	0.006 (0.002–0.013)	58/58 (100%)	10.01 (4.89–18.13)	0.70 (0.52–0.83)	< 0.0001	
HPV11	0	4/58 (7%)	0.000 (0.000–0.000)	7/57 (12%)	0.000 (0.000–0.000)	0.56 (–0.03–0.87)	< 0.0001	
	7	58/58 (100%)	0.058 (0.029–0.199)	57/57 (100%)	106.0 (56.60–158.0)	0.54 (0.30–0.75)	< 0.0001	0.92 (0.85–0.98)
	43	57/58 (98%)	0.007 (0.003–0.016)	58/58 (100%)	10.72 (5.24–17.93)	0.64 (0.41–0.79)	< 0.0001	
HPV16	0	7/58 (12%)	0.000 (0.000–0.000)	10/57 (18%)	0.000 (0.000–0.000)	0.76 (0.51–0.96)	< 0.0001	
	7	58/58 (100%)	0.184 (0.077–0.552)	57/57 (100%)	291.0 (178.0–468.0)	0.57 (0.35–0.75)	< 0.0001	0.93 (0.87–0.99)
	43	58/58 (100%)	0.019 (0.008–0.045)	58/58 (100%)	32.17 (13.32–64.67)	0.72 (0.54–0.85)	< 0.0001	
HPV18	0	6/58 (10%)	0.000 (0.000–0.000)	21/57 (37%)	0.000 (0.000–0.440)	0.36 (0.002–0.64)	0.0056	
	7	56/58 (97%)	0.058 (0.031–0.274)	57/57 (100%)	102.0 (44.00–210.0)	0.68 (0.49–0.80)	< 0.0001	0.69 (0.56–0.81)
	43	52/58 (90%)	0.005 (0.002–0.015)	58/58 (100%)	6.54 (2.07–17.35)	0.78 (0.64–0.86)	< 0.0001	
HPV31	0	4/58 (7%)	0.000 (0.000–0.000)	8/57 (14%)	0.000 (0.000–0.000)	0.63 (0.37–0.87)	< 0.0001	
	7	58/58 (100%)	0.145 (0.050–0.573)	57/57 (100%)	195.0 (118.0–541.0)	0.68 (0.48–0.82)	< 0.0001	0.85 (0.77–0.94)
	43	55/58 (95%)	0.016 (0.004–0.036)	58/58 (100%)	21.28 (10.48–49.17)	0.77 (0.60–0.87)	< 0.0001	
HPV33	0	3/58 (5%)	0.000 (0.000–0.000)	5/57 (9%)	0.000 (0.000–0.000)	0.79 (0.46–1.00)	< 0.0001	
	7	58/58 (100%)	0.238 (0.078–0.573)	57/57 (100%)	322.0 (197.0–677.0)	0.63 (0.40–0.79)	< 0.0001	0.93 (0.87–0.99)
	43	56/58 (97%)	0.020 (0.004–0.051)	58/58 (100%)	31.92 (13.36–69.07)	0.82 (0.71–0.89)	< 0.0001	
HPV45	0	2/58 (3%)	0.000 (0.000–0.000)	7/57 (12%)	0.000 (0.000–0.000)	0.53 (0.32–0.83)	< 0.0001	
	7	55/58 (95%)	0.202 (0.061–0.586)	57/57 (100%)	336.0 (140.0–562.0)	0.71 (0.51–0.83)	< 0.0001	0.87 (0.79–0.95)
	43	52/58 (90%)	0.016 (0.003–0.050)	58/58 (100%)	16.37 (8.63–63.59)	0.79 (0.60–0.88)	< 0.0001	

Type	Months	First-void urine		Serum		First-void urine vs Serum concentration		First-void urine vs Serum detection
		Antibody detection	Antibody concentration (AU-IU/ml or µg/ml)	Antibody detection	Antibody concentration (AU-IU/ml or µg/ml)	Spearman Correlation (95% CI)	Adjusted p-value	Cohen's Kappa (95% CI)
HPV52	0	0/58 (0%)	0.000 (0.000–0.000)	11/57 (12%)	0.000 (0.000–0.000)	NA	NA	
	7	57/58 (98%)	0.110 (0.035–0.507)	57/57 (100%)	297.0 (151.0–600.0)	0.65 (0.45–0.80)	< 0.0001	0.76 (0.66–0.87)
	43	49/58 (84%)	0.017 (0.003–0.046)	57/58 (98%)	28.27 (11.45–65.31)	0.80 (0.68–0.87)	< 0.0001	
HPV58	0	4/58 (7%)	0.000 (0.000–0.000)	9/57 (16%)	0.000 (0.000–0.000)	0.70 (0.38–0.90)	< 0.0001	
	7	58/58 (100%)	0.359 (0.079–0.849)	57/57 (100%)	365.0 (222.0–790.0)	0.64 (0.44–0.77)	< 0.0001	0.83 (0.74–0.92)
	43	53/58 (91%)	0.026 (0.010–0.078)	58/58 (100%)	39.81 (15.65–97.34)	0.81 (0.68–0.89)	< 0.0001	
Total IgG	0	58/58 (100%)	23.77 (16.57–56.07)	58/58 (100%)	6,660 (5,843–9,262)	0.23 (0.14–0.31)	< 0.0001	
	7	58/58 (100%)	22.40 (9.79–45.29)	57/57 (100%)	4,958 (3,505–6,599)	–0.014 (–0.11–0.08)	0.75	
	43	58/58 (100%)	23.21 (12.50–38.03)	57/57 (100%)	4,717 (3,870–7,108)	0.074 (–0.009–0.15)	0.092	

Data are median (IQR) or n (%). One volunteer did not provide consent to test serum samples at M0 and M7. Spearman rank correlation coefficients between first-void urine and serum samples at all time points were calculated. Kappa values between the antibody detection results in first-void urine and serum were calculated. Total IgG concentrations are reported in µg/ml for both sample types. AU=arbitrary units (HPV6, 11, 31, 33, 45, 52, 58). IU=international units (HPV16, 18). NA=not applicable.