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Human papillomavirus detection in urine: Effect of a first-void urine collection device and timing of collection

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ABSTRACT

Great interest has been directed towards the use of first-void (FV) urine as a liquid biopsy for high-risk HPV DNA testing. The aim of this study was to investigate the potential effect of a first generation FV urine collection device on the detection of HPV DNA and to assess if the concentration of HPV DNA varies between FV urine collected in the morning and those collected later during the day. In this prospective cohort study, 33 self-reported HPV-positive women participated. An FV urine sample was collected by these women in the morning (first urine of the day) and another sample was collected later that day for four consecutive days using two different collection methods; i.e., the Colli-Pee® and a standard urine cup. Samples were collected at home and returned at ambient temperature to the laboratory by postal mail. HPV DNA testing was conducted with the Riatol qPCR HPV genotyping assay. Based on the combined generalized linear mixed model used, there was no significant impact of the timing of collection (morning versus later during the day) on copies of HPV DNA, whereas Colli-Pee® collected samples show higher HPV concentrations than cup collected samples. However, at high concentrations of hDNA, the benefit of the Colli-Pee® disappeared.

1. Introduction

1.1. Background

The use of urine as liquid biopsy for human papillomavirus (HPV) DNA testing was shown to be very promising because of the high correlation between urinary and cervical HPV DNA, the ease of sampling and higher acceptability by women (Burroni et al., 2015; Combita et al., 2016; Ducancelle et al., 2014; Enerly et al., 2013; Franceschi et al., 2016; Hagihara et al., 2016; Leeman et al., 2017; Lorenzi et al., 2018; Nilyanimit et al., 2017; Pathak et al., 2014a; Payan et al., 2007; Sahasrabuddhe et al., 2014; Van Keer et al., 2018; Vorsters et al., 2012, 2016). Nevertheless, the standardization of urine collection, storage and processing techniques remain a major challenge (Pathak et al., 2014a; Vorsters et al., 2012). Hence, before possible implementation of urine HPV testing in screening, treatment and vaccination programmes, further evaluation and standardization is needed (Vorsters et al., 2014a).

The rationale for identifying HPV DNA in urine from women with a cervical or vaginal HPV infection is based on the fact that the cervicovaginal cavity, like all human mucosal cavities, is exfoliated and self-cleaned by the discharge of mucus or so-called vaginal smear. This smear accumulates between the labia minora, around the urethral opening. Upon initiation of urination this cervicovaginal discharge is washed away with the first part of the urine void (defined as *first-void* (FV) urine). This explains why the first collected part of a urine void contains significantly more HPV DNA than the subsequent part, as concluded by Pathak et al. in their meta-analysis (Pathak et al., 2014a). Previously published research confirms these findings, indicating that FV urine contains significantly more human DNA (hDNA) and HPV DNA than the subsequent fractions (Vorsters et al., 2014a). Based on the importance of the FV urine and the use of a preservative for HPV

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detection, a collection device (Colli-Pee®, PCT/EP2013/065853) was developed that allows immediate mixing of the FV urine with a preservation medium, while the rest of the urine void exits the device unhindered (avoiding the need to interrupt the stream).

Currently there remains some confusion regarding the definition of FV urine, which should refer to the initial stream of urine but is sometimes defined as the first urine of the day (Vorsters et al., 2014b). Nevertheless, in addition to the use of the FV urine, the timing of collection may also impact the amount of viral DNA in the sample, since more HPV DNA could be present when the interval between two urinations increases, as more excreted mucus and debris from the female genital organs has time to accumulate.

1.2. Objectives

With the aim to further standardize the detection of HPV DNA from urine samples, we conducted a prospective cohort study that was designed to (i) investigate the potential effect of a first generation FV urine collection device on HPV and hDNA concentration and to (ii) assess whether the concentration of HPV and hDNA varies between FV urine collected in the morning (first urine of the day) and FV urine collected later during the day.

2. Materials and methods

2.1. Study population and sample collection

Woman (aged 18–65 years) who had a previous HPV DNA-positive, clinician-taken, cervical sample were invited to participate. In total, 33 women were included and received a package via postal mail containing four Colli-Pee® devices (ref Colli-Pee CP-2000 series, Novosanis, Belgium) and four standard urine cups (ref A11259, Novolab, Belgium) to provide FV urine samples (July 2014 to February 2016) (Fig. 1). The women were asked to sample the very first FV urine of the day (U1) as well as an FV urine sample later during the day (U2) for four consecutive days. So, for each woman, a total of eight FV urine samples were obtained. All samples were taken in a prescribed randomized order, alternating the collection methods to eliminate potential bias associated with sampling order. An informational letter and instructions for use accompanied the devices. Women were requested to not extensively wash their genitals before FV urine collection and to not urinate at least one hour prior to the collection. The Colli-Pee® device allowed for capturing a fixed volume of 13 ml (+/- 2 ml) guaranteed FV urine and immediate mixing with 7 ml preservative (urine conservation medium (UCM)) leading to a final volume of 20 ml (+/- 2 ml), while the standard urine cup collected a variable volume of FV urine and required a manual transfer of 8 ml of urine to the 15-ml vial with 4 ml of UCM preservative by the participant. The FV urine samples were returned at ambient temperature by postal mail to the University of Antwerp. The returned samples were stored at −80 °C until processing. The Institutional Review Board (IRB) of the Antwerp University Hospital/University of Antwerp approved this clinical study, and participants signed informed consent before ensuing any study related procedures. To ensure patient confidentiality, each study participant received a personal identifier number to which all data were linked.

2.2. In-house DNA extraction from FV urine samples

FV urine samples were thawed after storage at −80 °C and DNA extraction was performed with an in-house protocol (Vorsters et al., 2014a). Briefly, in order to concentrate all DNA, including cell free DNA, 4 ml of the UCM-buffered FV urine aliquot was centrifuged at 3820 *g* for 20 min at 20 °C in an Amicon Ultra-4 50 K filter device (Merck Millipore, Belgium). NucliSENS Lysis Buffer (2 ml) (bioMérieux Benelux, Belgium) was added to the concentrate retained on the filter and incubated for 10 min at ambient temperature. All material was subsequently transferred to the NucliSENS Lysis buffer vial, and DNA extraction was performed on the NucliSENS® EasyMag® (bioMérieux) using the off-board lysis protocol. DNA was eluted in 55 µl, and 35 µl was transferred to a second vial with elution buffer (BioMérieux, Benelux) to reach a total volume of 75 µl of DNA extract used for HPV DNA genotyping.

2.3. HPV detection, genotyping and quantification

All FV urine DNA extracts were genotyped using the Riatol qPCR HPV genotyping assay. This assay quantifies beta-globin and 12 high-risk (hr) (HPV16/18/31/33/35/39/45/51/52/56/58/59), one probable high-risk (phr) (HPV68), three possible high-risk (pohr) (HPV53/66/67), and 2 low-risk (lr) (HPV6/11) HPV genotypes as described elsewhere (Depuydt et al., 2016). The results of hDNA and HPV DNA are reported in ng and copies per microliter of DNA extract for each genotype separately. The positivity threshold was set at 0.0001 HPV copy number per cell. HPV types were classified using the IARC Monograph Working Group Classification (IARC, 2016).

2.4. Statistics and software

The collected type specific HPV DNA concentrations have a skewed distribution with an abundance of measurements at zero. Because the probability of a nonzero outcome and the level of the nonzero outcome might be related, we combined a logistic regression model for the occurrence of a nonzero outcome with a log-linear regression model for the nonzero outcome values. Because multiple observations were taken for each patient, the data are correlated and we allowed for random intercepts for both models, which we assumed to be normally distributed and allowed to be correlated (Tooze et al., 2002). This combined model can be presented as follows:

$$P(Y_{ij} = 0|\theta_1) = 1 - p_{ij}(\theta_1)$$

$$P(Y_{ij} > 0|\theta_1) = p_{ij}(\theta_1)$$

$$[Y_{ij}|Y_{ij} > 0] = S_{ij}|\theta_2$$

with

$$logit(p_{ij}(\theta_1)) = \boldsymbol{X_{1ij}}\boldsymbol{\beta_1} + u_{1i}$$

$$\log(S_{ij}|\theta_2) \sim N(\boldsymbol{X_{2ij}}\boldsymbol{\beta_2} + u_{2i}, \sigma_e^2)$$

where Y_{ij} is defined as the HPV concentration for participant i at time j, $\theta_1 = [\boldsymbol{\beta_1}', u_{1i}]'$ is a vector of fixed and random effects and $\boldsymbol{X_{1ij}}$ is a vector of covariates for occurrence of a nonzero value, $\theta_2 = [\boldsymbol{\beta_2}', u_{2i}]'$ is a vector of fixed and random effects, $\boldsymbol{X_{2ij}}$ is a vector of covariates and σ_e^2 is the residual variance for the concentration of HPV DNA. Because measurements for the same HPV type were expected to be more alike than observations from different HPV types, HPV type was included in both models as a fixed effect. Additional fixed effects that were included in the starting model are method (cup or Colli-Pee®), timing (U1 or U2) and hDNA (ln(hDNA)), together with all two-way interaction terms (method*timing, method*hDNA and timing*hDNA). The final model was obtained using backwards model building (significance level 0.05). The models were fitted using SAS/STAT® (proc nlmixed). HPV positivity refers only to positivity of at least one of the 18 HPV types detected by the Riatol qPCR HPV genotyping assay.

3. Results

Repeated urine based self-sampling was performed by 33 women with a median age of 29 years (interquartile range (IQR): 27–37). A total of 258 urine samples were collected. For 2 out of the 33 women, a complete set of urine samples was not available for analysis (Fig. 1). One participant only had Colli-Pee® collected samples, while another

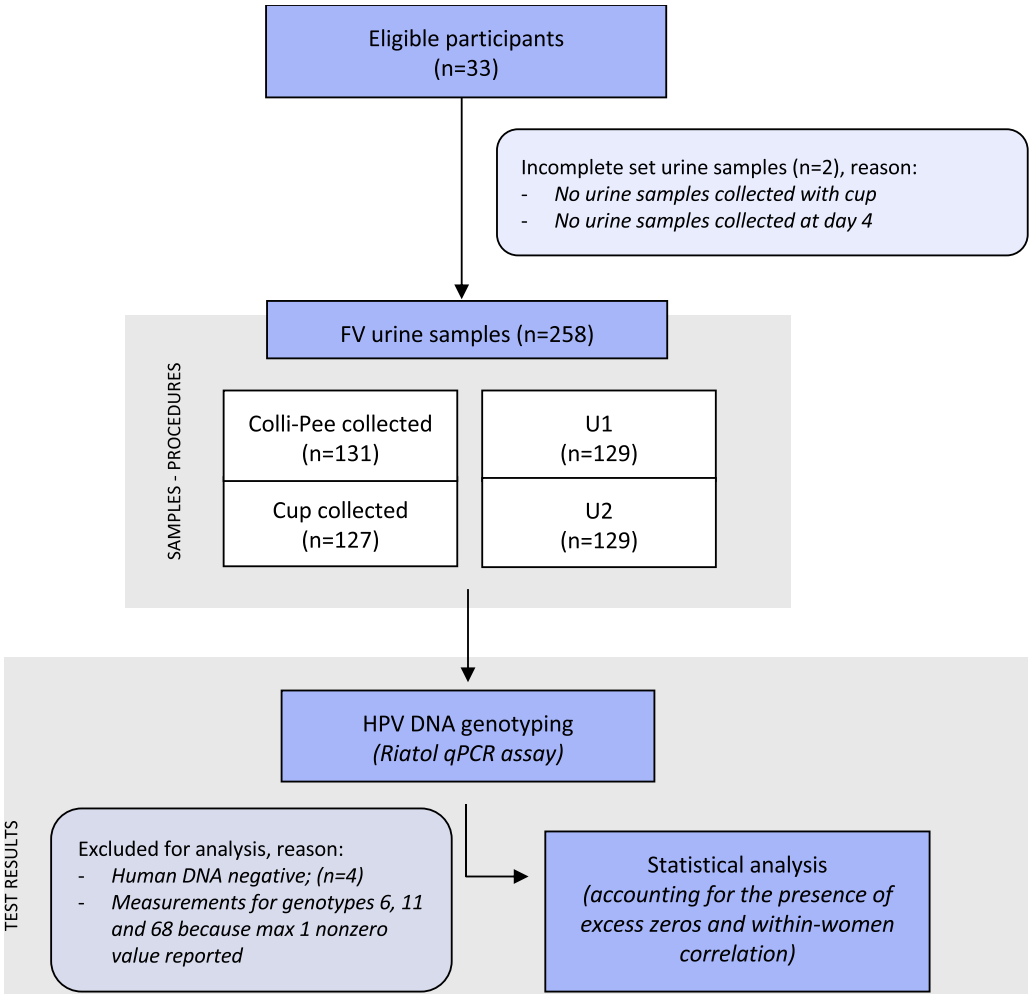

Fig. 1. ***Flow diagram of the study***. All 33 eligible participants received via postal mail four Colli-Pee® devices and four urine cups to provide FV urine samples. The women sampled the very first FV urine of the day (U1) and a sample later during the day (U2), for four consecutive days. One participant only provided Colli-Pee® collected samples and one only sample on three consecutive days. A total of 258 FV urine samples were collected (131/258 with the Colli-Pee® device and 129/258 were the first urine of the day). After HPV genotyping with the Riatol qPCR assay, four samples (2 collected with the Colli-Pee®) were negative for both hDNA (beta-globin) and HPV DNA and hence excluded from the model. Measurements for genotypes 6, 11 and 68 were excluded for statistical analysis because a maximum of 1 nonzero value was reported.

only sampled on 3 consecutive days. Four samples (from which 2 were collected with the Colli-Pee®) were negative for both hDNA (beta-globin) and HPV DNA, and hence excluded from the analysis. Measurements for HPV genotypes 6, 11 and 68 were excluded from statistical analysis because a maximum of one nonzero value was reported.

3.1. HPV genotype prevalence and concordance between urine samples

Concordant hrHPV positivity results during the sampling period were observed for 61% (n = 20/33) of the women, with either a daily presence of (p)hrHPV (n = 12/33) or no hrHPV at all during the sampling period (n = 8/33). The median hDNA yield in FV urine was respectively 7.14 (IQR: 2.87–17.85) and 4.5 (IQR: 1.88–9.15) ng per µl DNA extract for the Colli-Pee® device and urine cup, respectively. Among the 12 women that were consistently hrHPV positive during the sampling period, two were infected with only one HPV type. A maximum of four (p)hrHPV types were simultaneously detected in FV urine samples. Overall 119 hrHPV infections were detected with the Colli-Pee® and 112 with the cup (Table 1).

3.2. Effects of timing of collection and collection method

Because the correlation between the random intercepts was non-significant it was removed from the model. Both random intercepts and effects for HPV type were retained. Parameter estimates for the fixed effects are given in Table 2. The final model included hDNA_1 (the odds of observing HPV DNA depends only on hDNA and type of HPV), hDNA_2, the collection method (Colli-Pee®) and the interaction between hDNA_2 and the collection method (Colli-Pee®). Timing was not a significant fixed effect and hence was removed from the model by backwards model building. From the final model, we conclude that the conditional odds of observing a nonzero value increased significantly with increasing hDNA concentration (OR [95% CI] 1.22 [1.07;1.37]). The model further showed a significant interaction between the sampling method used and hDNA on the concentration of HPV DNA in the FV urine. Colli-Pee® collected samples show to have higher HPV concentrations than cup collected samples at concentrations of hDNA up to 12.18 ng/µl. At higher concentrations of hDNA, the benefit of the Colli-Pee® disappears (Fig. 2). Within this study, 103/125 (82,4%) cup collected FV urine samples had hDNA concentrations lower than 12.8 ng/µl and might benefit from the Colli-Pee®. 19/31 (61%) women only had cup collected FV urine samples with hDNA concentrations lower than 12.8 ng/µl.

4. Discussion

Keynotes for improved HPV DNA detection in urine were recently summarized by our research team (Van Keer et al., 2017). Briefly, these include 1) collection of FV, rather than random or mid-stream urine, as the initial flow of urine collects most of exfoliated cellular debris from the vagina, cervix, and uterus (Vorsters et al., 2014b; Pathak et al., 2014b; Senkomago et al., 2016; Lim et al., 2017); 2) avoidance of DNA degradation through the use of a preservative in both urine collection and processing (Vorsters et al., 2014a); 3) sufficient volume of urine to allow subsequent sample concentration (Vorsters et al., 2014a) and 4) recovery of cell-free HPV DNA in addition to cell-associated DNA

Table 1
Detection of hrHPV types in FV urine collected withthe Colli-Pee vs. the cup.

ID	1	2	3	5	7	9	10	11	12	13	14	15	16	17	18	22	23	24	25	27	29	30	31	32	Tot
Colli-Pee	4	2	8	8	7	3	8	3	4	4	4	0	4	2	4	7	4	4	10	7	0	3	15	4	119
HPV16	4			4			4									4				4					20
HPV18			4																						4
HPV31			4	4	3				3	1	1							1	4						21
HPV33					4		4								4										12
HPV35						3		3					4												10
HPV39																3									3
HPV45																	2	1							3
HPV51																	2	1	2						5
HPV52											3								4	2			3	4	16
HPV56										3													4		7
HPV58									1					2									4		7
HPV59		2																		1		3	4		10
HPV68																		1							1
Cup	4	3	8	8	7	1	8	2	4	4	4	1	4	3	4	7	6	0	8	6	3	2	10	5	112
HPV16	4			4			4									4				3					19
HPV18			4							1															5
HPV31			4	4	3	1			4			1							4						21
HPV33					4		4								3										11
HPV35								2					4												6
HPV39																3								1	4
HPV45																	3								3
HPV51																	3				2				5
HPV52											4								4	2			3	4	17
HPV56										3													2		5
HPV58														3	1								1		5
HPV59		3																		1	1	2	4		11
HPV68																									

Table 2
Parameter estimates for the fixed effects in the final model. *We combined a logistic regression model[(a)] for the occurrence of a nonzero outcome with a* ***log****-linear regression model for the nonzero outcome values[(b)]. Because multiple observations were taken for each patient, the data are correlated, and we allowed for a random intercept for both models.*

	Parameter	Estimate	95% confidence interval	p-value
logistic regression model	**Intercept1**	−3.08	[-3.84; -2.32]	< 0.0001
	hDNA_1	0.22	[0.07; 0.37]	0.0064
log-linear regression model	**Intercept2**	11.01	[9.39; 12.64]	< 0.0001
	hDNA_2	1.39	[1.02; 1.76]	< 0.0001
	Method (Colli-Pee®)	1.26	[0.34; 2.17]	0.0086
	hDNA_2*method (Colli-Pee®)	−0.48	[-0.86; -0.10]	0.0155

(Vorsters et al., 2014a). With the general aim of further standardizing the detection of HPV DNA in FV urine samples, this study was designed to investigate the potential effect of a first generation FV urine collection device (Colli-Pee®) on HPV concentration in FV urine. In addition, we assessed the effect of time of collection on the concentration of HPV; i.e., first urine of the day and a sample collected at a later time during the day.

4.1. Main findings

The timing of collection did not have a significant effect on the number of copies of DNA in FV urine samples for both hDNA and HPV DNA. Similar HPV DNA concentrations were detected in FV urine collected in the morning compared to that of FV samples collected later during the day, suggesting there is no advantage in testing morning FV urine over FV urine that was collected later during the day. Our findings presented here confirm these reported by (Leeman et al. (2017)). The fraction of the urine appears to be more important than the timing, which is in agreement with results from a recent study by Senkomago

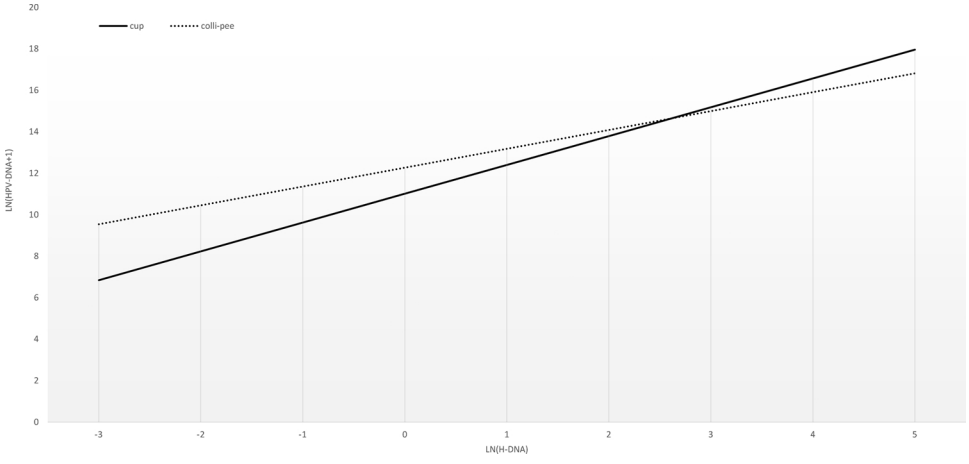

Fig. 2. ***Graphical representation of the impact of the interaction term between the concentration of human DNA (ln(ng/µl) and the method of collection on the concentration of HPV DNA (ln(copies/µl DNA extract +1)).*** *Colli-Pee® collected samples have higher HPV concentrations than cup collected samples at hDNA concentrations up to 12.18 ng/µl. At higher concentration of hDNA the benefit of the Colli-Pee® disappears.*

et al. comparing different fractions and collection times for the detection of hrHPV DNA and high-grade cervical precancerous lesions (Senkomago et al., 2016).

From the model we can conclude that the conditional odds of observing a nonzero value for HPV DNA increased significantly with increasing hDNA concentration. Furthermore, a significant interaction between the collection method used and hDNA on the number of copies of HPV DNA in FV urine was found. Colli-Pee® collected samples show higher HPV DNA concentrations than cup collected samples at hDNA concentrations up to 12.18 ng/µl. At higher concentrations of hDNA (more concentrated samples), the benefit of the Colli-Pee® disappeared. Overall, this interaction should be interpreted with caution because of the small number of observations. It would be of interest to see if this correlation also occurs in larger datasets and with other HPV assays. In a recent study by Tshomo et al. it was shown that HPV prevalence in FV urine varies according to the HPV assay sensitivity (Tshomo et al., 2017). Not considering hDNA as a fixed effect, we observed significantly higher HPV DNA concentrations in Colli-Pee® collected samples. Therefore, we expect to obtain a higher HPV prevalence in Colli-Pee® collected samples compared to when cup-collected FV urine samples are used, especially in combination with less sensitive HPV assays.

In this study, we have shown a moderate degree of consistency concerning positivity and negativity of HPV DNA in urine during four consecutive days of sampling. There were fluctuations in viral DNA copy numbers from day to day in all infected women, but overall the viral copy numbers per µl of DNA extract were generally sufficient for the detection of HPV in the FV urine samples. As in the study of (Senkomago et al. (2016)), we provide information on the delivery of urine collection kits by postal mail, which could inform researchers and health-care workers for future implementation. Similarly, as in the study by Sellors et al., women reported being comfortable with receiving a urine collection kit at home by postal mail (Sellors et al., 2000).

Notably, all but four urine samples contained detectable hDNA. However, hDNA is not an ideal confirmation of good sample storage or sample processing because HPV DNA may decay faster than hDNA (Vorsters et al., 2014a). However, hDNA-negative urine samples may indicate suboptimal sample collection, handling or extraction methods and result in a lower HPV prevalence (Vorsters et al., 2016, 2014a).

It is worth mentioning that in the beginning of this study, the male partners were asked to provide urine samples. Recent vaginal intercourse might affect HPV detection since false-positive tests could result from detecting another person's HPV DNA. However, we found this sampling method to be much less useful in men (*data not shown*). Indeed, the rationale for finding HPV DNA in FV urine from women is not transferable to males. The amount of HPV (if any) and hDNA in male FV urine was much lower compared to women. However, as mentioned by Koene et al., the addition of urine samples to penile swabs could be of use in epidemiological or clearance studies (Koene et al., 2016).

Briefly, the rationale for identifying HPV DNA in urine is that upon initiation of urination most cervicovaginal discharge is washed away with the first part of the urine void. The women in this study were asked to provide, in addition to the Colli-Pee®, an FV urine sample with a standard urine cup. We can conclude from the model that an FV urine collection device may help to enhance and standardize HPV DNA detection in FV urine. This standardization is possible since the Colli-Pee® is placed against the body by the women before initiation of urination and allows for capturing a fixed volume of guaranteed FV urine (without interrupting the urine flow), so likely less errors in sampling. In addition, the Colli-Pee® allows for immediate mixing with preservative, so likely less DNA degradation. However, since we did not ask the women to record the start volume in the urine cup before transferring the FV urine to the 15-ml vial, we cannot determine equal or better detection when capturing the same amount or even less FV urine as the Colli-Pee® device. Indeed, we expect to get better HPV DNA results when using more concentrated samples, i.e. lower volume. Further studies are warranted to explore different volumes of FV urine, which could be important in further maximization of the detection of HPV DNA or other biomarkers. An additional interesting route to further explore is determining to what extent the non-invasive character of urine sampling brings added value in monitoring the natural history of an HPV infection. Indeed, urine sampling does not interfere with the natural history of the infection. In contrast, cellular sampling by scraping the epithelium with a cytobrush creates micro-lesions that may allow for new infections (Roberts et al., 2011, 2007) - if infectious virus particles are present - or potentially induce an inflammatory reaction, perhaps clearing the infection.

4.2. Limitations of the study

The findings of this study need to be considered in the context of the potential limitations, including the relatively small sample size. It would be of interest to see if the same conclusions can be drawn in larger studies and with other HPV assays. Second, the results have no clinical outcome to correlate with, however, most of the women in the cohort were followed up without requiring any immediate intervention. In addition, no information was available about the time between the woman's last self-reported HPV positive sample and the start of her sampling period within the study. Because of this selected population of women, the results cannot be directly extrapolated to other populations. The Colli-Pee® results should be interpreted with caution, as the device used for this study was not the latest generation Colli-Pee® device (FV-5000 series) that is now on the market. The device consisted of seven components including a cardboard funnel and a commercially available polystyrene Sterilin tube. The main difference between the two generations resides in its usability and the improved proprietary tube design that allows for improved storage and shipment conditions. However, there is no difference in the mechanism of action or collected FV urine volume. Additionally, in previous studies it has been suggested that the menstrual cycle might affect the amount of vaginal hrHPV in a sample. However, the results of these studies are somewhat conflicting (Sanner et al., 2015; Schneider et al., 1992; Sherman et al., 2006). In our study, no information was gathered concerning the menstrual cycle.

5. Conclusion

In conclusion, the results of the present study provide further evidence for the notion that FV urine is a reliable sample for HPV DNA testing when using an appropriate preservative and DNA extraction method in combination with a PCR-based HPV test. FV urine collected in the morning (the first urine of the day) did not contain a higher HPV DNA concentration than FV urine collected later during the day. Furthermore, the final model indicates that Colli-Pee® collected samples show higher HPV concentrations than cup collected samples at hDNA concentrations up to 12.18 ng/µl. At higher concentrations of hDNA, the benefit of the Colli-Pee® disappeared. Within this study, 103/125 (82,4%) cup collected FV urine samples had hDNA concentrations lower than 12.8 ng/µl and might benefit from the Colli-Pee®. 19/31 (61%) women only had cup collected FV urine samples with hDNA concentrations lower than 12.8 ng/µl.

Declaration of interest

Novosanis, that produces and provided the Colli-Pee® device, is a spin-off company of the University of Antwerp. VA, VV, BK, and VDP are co-founders and board members of Novosanis.

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Appendix A

See Tables A1–A3.

Table A1
Prevalence of HPV during the sampling period of each woman. *The range of (p)hrHPV copies (log copies/μl DNA extract) and hDNA copies (ng/μl) according to collection method. ((p)hrHPV; (probable) high-risk HPV; [a]incomplete set urine samples; [b]samples negative for hDNA).*

ID	HPV types detected	hDNA copies range *(ng/μl)*		(p)hrHPV types *(times detected)*	(p)hrHPV copies type range *log(copies/μl DNA extract)*		HPV +/- consistent (%)	hrHPV consistent (%)
		Colli-Pee®	Cup		Colli-Pee®	Cup		
1	16	1.61-11.1	0.5-1.7	HPV16 (8)	6.18-7.74	4.31-5.12	100	100
2	59/67	6.61-15.43	4.16-12.11	HPV59 (5)	4.30-4.82	4.62-5.01	88	63
3	18/31	0.94-6.31	0.96-3.82	HPV18 (8)	4.18-5.60	2.25-5.77	100	100
				HPV31 (8)	4.70-6.44	3.27-5.46		
4[a]	58	0.28-7.68	–	HPV58 (4)	6.46-8.75	–	100	100
5	16/31/53	5.09-16.85	1.64-4.9	HPV16 (8)	8.66-10.08	7.68-8.41	100	100
				HPV31 (8)	8.51-9.39	6.99-8.28		
6	Neg	1.25-2.48	0.94-3.83	–	–	–	100	–
7	31/33	0.21-3.16	0.13-1.62	HPV31 (6)	0.57-4.38	1.97-4.66	100	100
				HPV33 (8)	2.71-5.50	1.53-5.26		
8[b]	Neg	0.06-3.53	1.25-2.19	–	–	–	100	–
9	31/35/53/67	1.45-34.17	9.03-19.44	HPV31 (1)	–	2.81	100	50
				HPV35 (3)	8.06-8.34	–		
10	16/33	6.4-13.11	4.87-10.45	HPV16 (8)	7.93-8.84	7.62-8.87	100	100
				HPV33 (8)	7.31-7.96	7.06-8.08		
11	35	4.53-11.43	3.67-6.64	HPV35 (5)	7.73-8.00	7.26-7.51	63	63
12	31/53/58	0.61-17.98	0.17-15.33	HPV31 (7)	5.74-6.75	1.91-5.42	88	88
				HPV58 (1)	5.46	–		
13	11/18/31/56/66	2.27-20.19	1.71-5.2	HPV18 (1)	–	6.30	88	88
				HPV31 (1)	3.85	–		
				HPV56 (6)	3.29-5.51	2.81-5.02		
14	31/52/67	1.09-4.95	0.93-3.37	HPV31 (1)	3.92	–	100	100
				HPV52 (7)	6.98-8.15	6.53-8.10		
15	31	1.75-11.27	3.96-12.15	HPV31 (1)	–	4.24	88	13
16	35/53	1.35-5.46	1.72-3.91	HVP35 (8)	6.88-7.18	6.34-7.03	100	100
17	58/67	1.45-14.88	0.87-3.98	HPV58 (5)	8.60-9.22	7.62-8.15	75	63
18	33/53/58	3.27-5.58	0.38-3.98	HPV33 (7)	4.47-6.37	2.29-3.83	88	88
				HPV58 (1)	–	3.92		
19[b]	Neg	8.1-17.71	4.5-9.19	–	–	–	100	–
20	Neg	2.77-18.36	3.95-9.95	–	–	–	100	–
21	Neg	1.78-6.66	1.87-5.94	–	–	–	100	–
22	16/39/67	7.17-22.67	3.38-14.22	HPV16 (8)	6.61-7.62	5.59-7.06	100	100
				HPV39 (6)	7.15-7.70	6.92-7.41		
23[a]	45/51/53/66	14.84-99.72	4.8-13.71	HPV45 (5)	5.56-5.81	5.03-5.38	83	83
				HPV51 (5)	5.08-5.35	4.94-5.32		
24	31/45/51/53/68	1.72-142.62	2.99-9.49	HPV31 (1)	3.09	–	88	13
				HPV45 (1)	5.58	–		
				HPV51 (1)	5.77	–		
				HPV68 (1)	5.74	–		
25	31/51/52	25.95-73.9	6.28-109.39	HPV31 (8)	4.92-7.16	4.62-6.40	100	100
				HPV51 (2)	4.33-4.63	–		
				HPV52 (8)	5.56-6.40	5.74-5.97		
26	Neg	3.95-40.78	16.01-34.86	–	–	–	100	–
27	16/52/59/66/67	14.86-34.96	1.85-16.09	HPV16 (7)	3.87-4.43	4.03-4.60	88	88
				HPV52 (4)	5.74	5.74-5.83		
				HPV59 (2)	3.88	4.18		
28	Neg	16.79-27.43	6.21-18.84	–	–	–	100	–
29	51/59	6.44-61.52	0.98-14.57	HPV51 (2)	–	3.31	75	25
				HPV59 (1)	–	4.18		
30	53/59/66/67	6.36-14.44	1.2-8.3	HPV59 (5)	3.27-4.18	4.18-4.87	63	63
31	52/53/56/58/59/66/67	3.22-35.56	3.32-24.43	HPV52 (6)	5.97-6.13	5.74-6.32	100	100
				HPV56 (6)	4.63-5.38	4.63-4.99		
				HPV58 (5)	6.86-7.76	7.28		
				HPV59 (8)	5.11-6.51	3.89-5.72		
32	39/52	42.63-77.91	34.23-52.9	HPV39 (1)	–	6.68	100	100
				HPV52 (8)	5.74-6.92	6.32-6.77		
33[b]	Neg	0.5-1.67	0.29-1.89	–	–	–	100	–

Table A2

Consistency of HPV during the sampling period of each woman. *The range of (p)hrHPV copies (log copies/µl DNA extract) and hDNA copies (ng/µl) according to collection method. ((p)hrHPV; (probable) high-risk HPV; [a]Incomplete set urine samples; [b]samples negative for hDNA); na, not applicable.*

	(Colli-Pee/Cup)				*Colli-Pee hrHPV positive (%)*	*Cup hrHPV positive (%)*
	DAY1	**DAY2**	**DAY3**	**DAY4**		
ID1	+/+	+/+	+/+	+/+	100	100
ID2	+/-	-/+	+/+	-/+	50	75
ID3	+/+	+/+	+/+	+/+	100	100
ID4	+/na	+/na	+/na	+/na	100	na
ID5	+/+	+/+	+/+	+/+	100	100
ID6	negative				na	na
ID7	+/+	+/+	+/+	+/+	100	100
ID8	negative				na	na
ID9	+/-	+/-	-/+	+/-	75	25
ID10	+/+	+/+	+/+	+/+	100	100
ID11	+/+	-/+	+/-	+/-	75	50
ID12	+/+	+/+	+/+	-/+	75	100
ID13	-/+	+/+	+/-	+/+	75	75
ID14	+/+	+/+	+/+	+/+	100	100
ID15	-/-	-/-	-/+	-/-	0	25
ID16	+/+	+/+	+/+	+/+	100	100
ID17	+/+	+/+	-/+	-/-	50	75
ID18	+/+	+/+	+/+	+/-	100	75
ID19	negative				na	na
ID20	negative				na	na
ID21	negative				na	na
ID22	+/+	+/+	+/+	+/+	100	100
ID23	+/+	+/+	-/+	na	67	100
ID24	+/-	-/-	-/-	-/-	25	0
ID25	+/+	+/+	+/+	+/+	100	100
ID26	negative				na	na
ID27	+/-	+/+	+/+	+/+	100	75
ID28	negative				na	na
ID29	-/-	-/+	-/-	-/+	0	50
ID30	+/+	+/+	-/-	+/-	75	50
ID31	+/+	+/+	+/+	+/+	100	100
ID32	+/+	+/+	+/+	+/+	100	100
ID33	negative				na	na
	Total average (%)				79%	78%

Table A3

Number of women with cup collected FV urine samples with hDNA < 12.8 ng/µl.

Number of cup collected FV urine samples with hDNA < *12.8 ng/µl*	Number of women (#/%)	IDs
All	19/31	ID2,3,5,6,7,8,10,11,13,14,15,16,17,18,19,20,21,24,30
3/4	7/31	+ ID1,12,22,27,29,31,33
2/4	2/31	+ ID9,28
1/4	1/31	+ ID25
0/4	2/31	+ ID26,32

*ID23(1/3) - collected only for 3 consecutive days.

**ID4 - only Colli-Pee collected samples.

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