HPV DNA detection in urine: effect of a first-void urine collection device and timing of collection

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Objectives

To evaluate the use of a prototype first-void (FV) urine collection device (Colli-Pee™, Novosanis) and assess the effect of collection timing on the likelihood of detecting human and HPV DNA in women.

Methods

First-void (FV) urine collection

(self-reported prior HPV positive test)

n=33

Colli-Pee™ (Novosanis)

Urine cup

Collected in the morning (first urine of the day) and afternoon on 4 consecutive days at home, while alternating the collection method (total 8 FV urine samples/participant)

Amicon filtration & DNA extraction

Amicon filtration (Merck Millipore) and NucliSENS® easyMAG® DNA extraction (bioMérieux) [1]

HPV DNA genotyping

- In-house HPV 16 and human DNA TS qPCR at Uantwerp
- Riatol qPCR HPV genotyping assay- separate typing of hrHPV types 16,18,31,33,35,39,45,51,52,56,58,59,66,68; phr type 53 and lr types 6 & 11

*hrHPV: high risk human papillomavirus; phr: potentially high-risk; lr: low-risk

Results

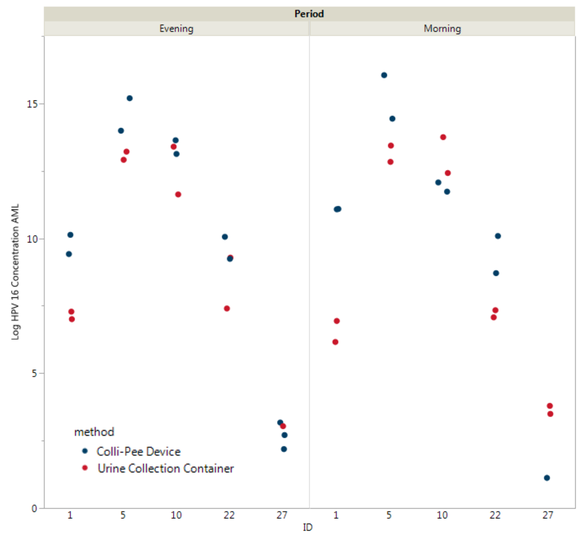
A sample of 33 women was analysed. For 13 women, all samples tested positive; for 8 women all samples were negative. 7 women had one out of eight samples negative, 2 had six out of eight samples positive and 3 had 2 or less of eight samples positive **(Fig 1).**

Fig 1. Schematic overview of HPV DNA results over the 4 day period. HPV DNA results are provided for each participant. Red: negative, green: positive. The top row indicates time-point of sampling and collection method. (fail: no hDNA detected; n/a: not provided or insufficient volume)

ID	Day 1		Day 2		Day 3		Day 4	
	Morning	Evening	Morning	Evening	Morning	Evening	Morning	Evening
	Colli-Pee	**Urine Cup**	**Urine Cup**	**Colli-Pee**	**Colli-Pee**	**Urine Cup**	**Urine Cup**	**Colli-Pee**
1	16	16	16	16	16	16	16	16
5	16/31/53	16/31/53	16/31/53	16/31/53	16/31/53	16/31/53	16/31/53	16/31/53
7	33	31/33	33	31/33	31/33	31/33	31/33	31/33
9	35/53/67	53/67	53/67	35/53/67	67	31/53/67	53/67	35/53/67
11	35	35	35	Neg	35	35	Neg	35
12	31	31	31	31	31/58	31	31/53/58	Neg
15	Neg	Neg	Neg	Neg	Neg	31	Neg	Neg
17	58	58	58	58	Neg	58	Neg	67
19	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
20	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
24	31/45/51/ 53/68	Neg	Neg	Neg	Neg	Neg	Neg	Neg
28	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
29	Neg	Neg	51	Neg	Neg	Neg	51/59	Neg
31	52/56/58/ 59/66	52/56/58/ 59/66	52/59/66	52/53/56/ 58/59/66	52/53/56/ 58/59/66	52/56/ 59/66	59/66/67	56/58/ 59/66
32	52	52	39/52	52	52	52	52	52
ID	**Urine Cup**	**Colli-Pee**	**Colli-Pee**	**Urine Cup**	**Urine Cup**	**Colli-Pee**	**Colli-Pee**	**Urine Cup**
2	Neg	59/67	67	59/67	59/67	59/67	67	59/67
4	n/a	58	58	n/a	n/a	58	58	n/a
6	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
8	fail	Neg	Neg	Neg	Neg	Neg	Neg	Neg
10	16/33	16/33	16/33	16/33	16/33	16/33	16/33	16/33
13	18/56/66	66	56/66	11/56/66	Neg	56	31/56	56/66
16	35	35	35	35	35	35	35	35/53
18	33	33	33	33/58	33	33	33/53	Neg
21	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
23	45/51/66	45/51/53	45/51/ 53/66	45/51/53	45/51/ 53/66	Neg	n/a	n/a
25	31/52	31/52	31/51/52	31/52	31/52	31/51/52	31/52	31/52
30	53/59/66	53/59/66	59	59/66	66	53/66	53/59/ 66/67	Neg
33	Neg	fail	Neg	Neg	Neg	Neg	Neg	fail
ID	**Colli-Pee**	**Urine Cup**	**Colli-Pee**	**Urine Cup**	**Urine Cup**	**Colli-Pee**	**Urine Cup**	**Colli-Pee**
3	18/31	18/31	18/31	18/31	18/31	18/31	18/31	18/31
22	16/39/67	16/39/67	16/39/67	16/39/67	16/39/67	16/67	16/67	16/39/67
ID	**Urine Cup**	**Colli-Pee**	**Urine Cup**	**Colli-Pee**	**Colli-Pee**	**Urine Cup**	**Colli-Pee**	**Urine Cup**
14	52	52	52	52	31/67	52	52	52
ID	**Colli-Pee**	**Urine Cup**	**Urine Cup**	**Colli-Pee**	**Urine Cup**	**Colli-Pee**	**Urine Cup**	**Colli-Pee**
26	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
ID	**Urine Cup**	**Colli-Pee**	**Urine Cup**	**Colli-Pee**	**Urine Cup**	**Colli-Pee**	**Colli-Pee**	**Urine Cup**
27	Neg	16	16/67	16	16/52/ 59/66	16/52	16/52/59	16/52

Fig 2. Influence of afternoon vs. first urine of the day and collection method of HVP16 and hDNA in HPV16 DNA positives (5/33) (Red: Urine Cup; Blue: Colli-Pee™ device)

a



b

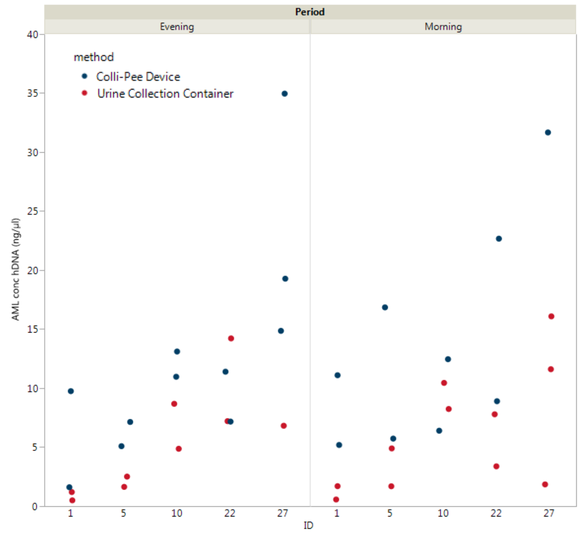


Fig 3. Ct (cycle threshold) values for the in house qPCR per participant. (Red: Urine Cup; Blue: Colli-Pee™ device)

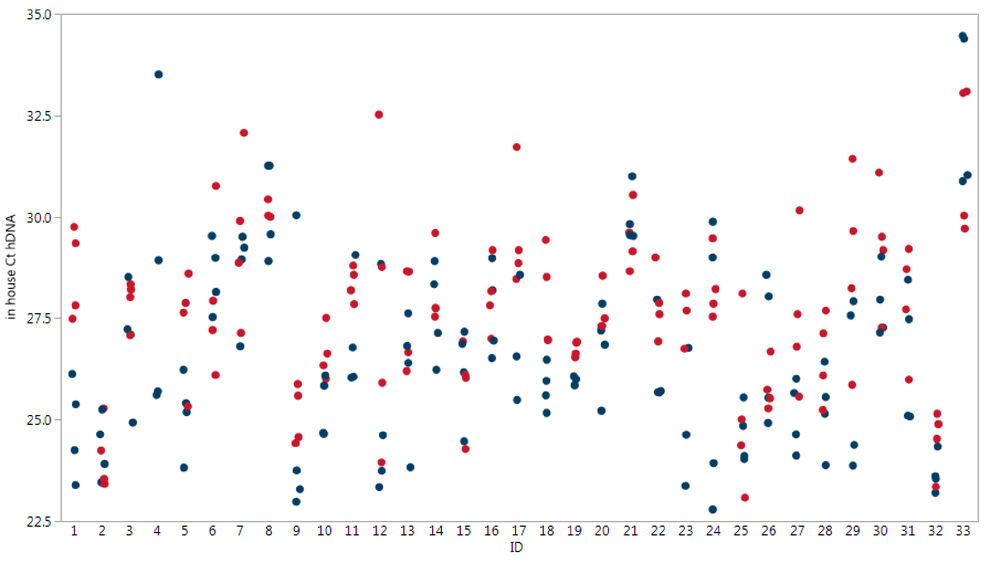


Fig 2a and **fig 2b** show no impact of timing of collection on copies hDNA and HPV16 in HPV16 positives. Whereas, it does show an impact of the collection method.

Fig 3 shows the in house Ct-hDNA values for every participant. After fitting a linear mixed model for the in house Ct-value of hDNA, a p-value of <0,0001 was found for method, whereas the p-value for period was not significant (p-value 0,6370)

Conclusion

- Consistent with previous studies [2], our study also demonstrates that when an appropriate preservative and DNA extraction method are used, FV urine is a reliable and reproducible sample for HPV DNA testing
- A FV urine collection device may help to enhance and standardize HPV DNA detection in urine, independent whether the FV urine is taken from the first urine of the day or from urine provided later in the day
- An intra- and inter participant variability was observed

References

1/ Vorsters, A., Van den Bergh, J., Micalessi, I., Biesmans, S., Bogers, J., Hens, A., De Coster, I., Ieven, M., and Van Damme, P. (2014). Optimization of HPV DNA detection in urine by improving collection, storage, and extraction. Eur J Clin Microbiol Infect Dis 33, 2005-2014.

2/ Vorsters, A., Van Damme, P., Clifford G. (2014). Urine testing of HPV: rationale for using first void. BMJ, 349:g5264

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