HPV DNA detection in urine: Effect of a first-void urine collection device and time of collection

Alex Vorsters[1], Severien Van Keer[1], Samantha Biesmans[1], Vanessa Vankerckhoven[1,2], Manon de Koeijer[2], Koen Beyers[2], Margareta Ieven[1,3], Pierre Van Damme[1]
[1]University of Antwerp, VAXINFECTIO, Belgium; [2]Novosanis, Wijnegem, Belgium; [3]Antwerp University Hospital, Belgium.

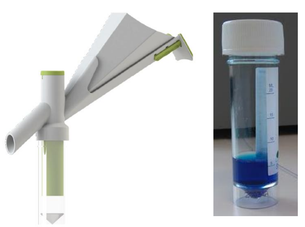
Introduction and objectives:

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

Methods:

Participants with a self-reported HPV infection were asked to provide eight FV urine samples (four FV first of the day urine samples and four FV evening urine samples) over a period of four days. Two FV urine collection methods were alternated, i.e. the Colli-Pee™ device and a collection set with standard urine cup and a pipet for transfer of urine. Both methods used a preservative (UCM) pre-filled vial. Urine samples were collected at home and sent by mail to the lab. Human DNA quantification as well as HPV DNA detection, genotyping, and quantification were performed on all samples [1]. hDNA and HPV16 quantification were performed in house, HPV genotype detection and quantification of other genotypes was performed with real-time type specific (TS) PCR at RIATOL, Antwerp, Belgium. P values are calculated using Related Samples Wilcoxon Signed Rank Test in SPSSv22 software.

Fig 1: Sample collection and processing of samples.



Two collection methods

Colli-Pee™: Allows capturing fixed volume of first void urine and immediate mixing with preservative buffer/dye.

Urine cup: Variable volume of first-void urine and manual transfer to vial with preservative buffer/dye.

Samples of 22 women collected in the morning (first urine of the day) and afternoon on 4 consecutive days, while alternating the collection method, were analyzed (n = 8 samples/women * 22 women = 176 samples).

Manual Amicon Ultra filtration: 4mL loaded on filter (Merck Millipore)

NucliSENS® easyMag® extraction (bioMérieux)

In house HPV16 and hDNA real-time PCR

TS real-time PCR at RIATOL

Results :

Complete and correctly labelled sample sets from 22 women were analyzed. All samples tested positive for 14 women; all eight samples were negative for 4 women. Two women had one out of eight samples negative and one women had six out of eight samples negative.
Fig 2a, Fig 3a and Fig 4a show no impact of time of collection on copies of HPV16, copies of all detected HPV genotypes and copies of human DNA, respectively. Whereas Fig 2b, Fig 3b and Fig 4b clearly show a significant impact of the collection method.
Fig 5 shows that there is a patient specific correlation between the copies hDNA and HPV16 found in urine.

Discussion:

The hypothesis for finding HPV DNA in urine of women with a cervical HPV infection is that, during urination, urine gets contaminated by debris and impurities lining the urethra opening, including mucus and debris of exfoliated cells from the vagina, cervix and uterus. It hence follows that the initial flow of urine collects most of this debris [2]. These results confirm that the Colli-Pee is able to capture more efficiently this first part of the urine flow compared to a standard urine cup, leading to a more concentrated sample.

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Fig 2a: Boxplot showing the influence of afternoon or first urine of the day collection in HPV 16 DNA positives (each box represents 4 samples collected over 4 days).

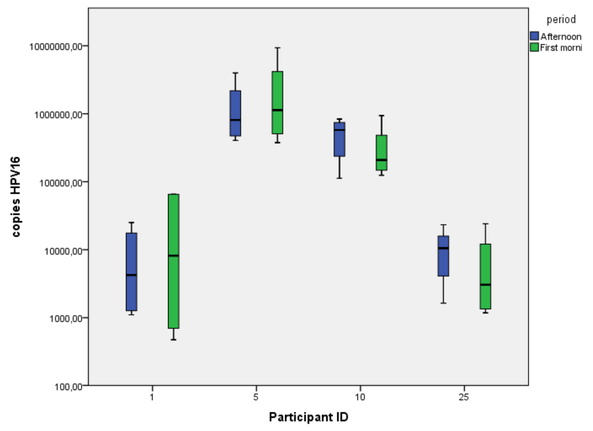


Fig 2b: Boxplot showing the influence of the collection method in HPV 16 DNA positives (each box represents 4 samples collected over 4 days from one participant).

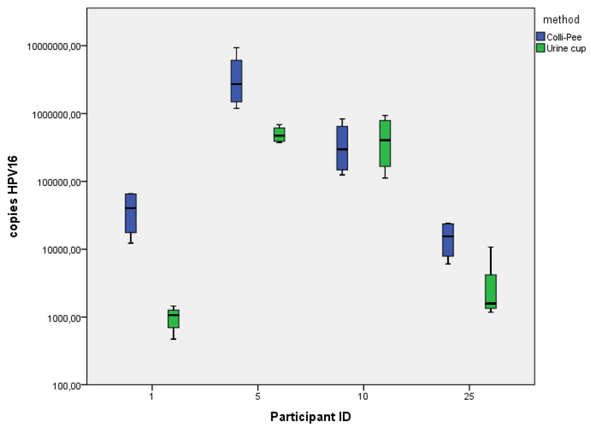


Fig 3a: Average copies of HPV DNA per participant and per HPV genotype found in first morning FV and afternoon FV urine.

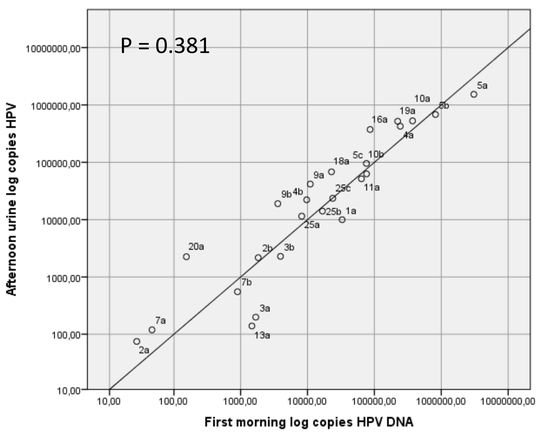


Fig 3b: Average copies of HPV DNA per participant and per HPV genotype found in Colli-Pee and Urine cup collected FV urine.

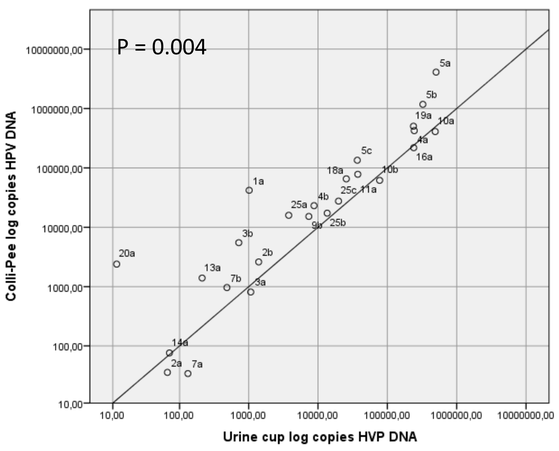


Fig 4a: Average copies of hDNA per participant found in first morning FV and afternoon FV urine..

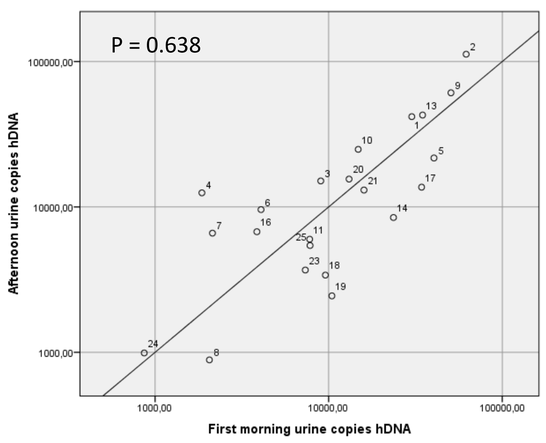


Fig 4b: Average copies of hDNA per participant found in Colli-Pee and Urine cup collected FV urine.

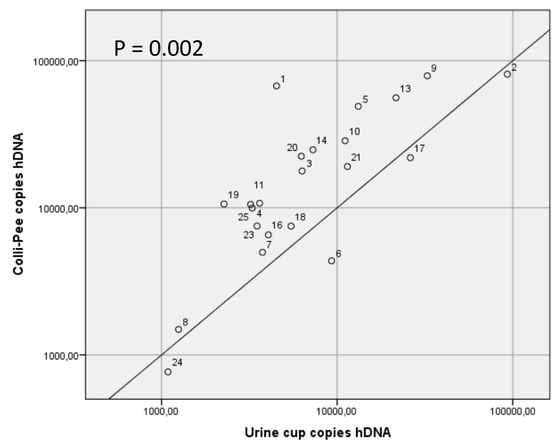
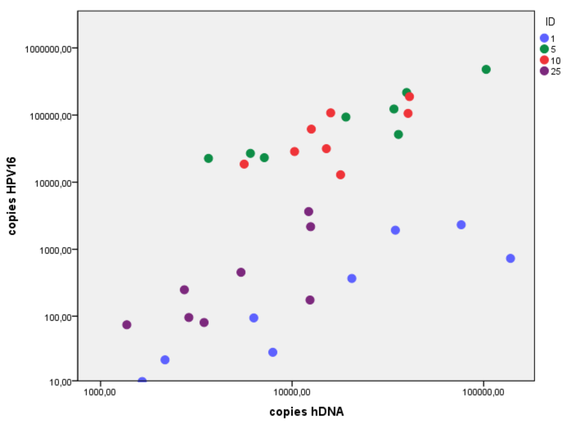


Fig 5: Correlation between copies hDNA and HPV 16 DNA in FV urine.



Conclusion:

These results further confirm that when an appropriate preservative and DNA extraction method is used, urine is a reliable and reproducible sample for HPV DNA testing.
A FV urine collection device may help to enhance HPV DNA detection in urine, independent whether the FV is taken from the first urine of the day or from urine provided later in the day.

References:

1) A. Vorsters, J. Van den Bergh, I. Micalessi, S. Biesmans, J. Bogers, A. Hens, I. De Coster, M. Ieven, P. Van Damme. Optimization of HPV DNA detection in urine by improving collection, storage, and extraction. Eur J Clin Microbiol Infect Dis. 2014
2) Vorsters A, Van Damme P, Clifford G. Urine testing for HPV: rationale for using first void. BMJ, 2014;349:g5264.

Contact :
alex.vorsters@uantwerpen.be