# Pilot feasibility study on use of Cepheid Xpert®HPV with Colli-Pee™ collected UCM preserved urine.

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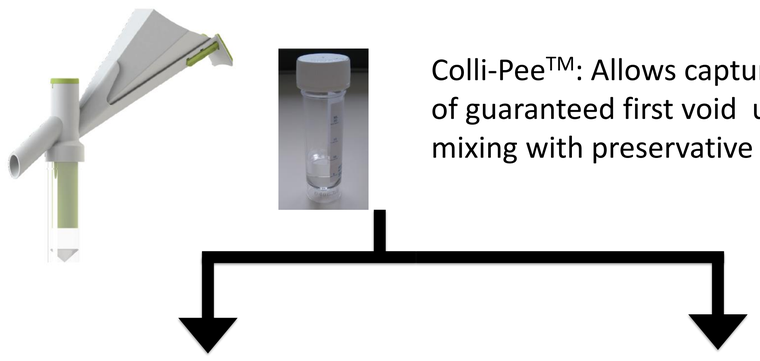
**Introduction and objectives:**

- HPV testing in urine has been proposed for monitoring impact of vaccination, follow-up of treatment and/or reaching women not participating in cervical cancer screening programs.
- The use of Colli-Pee™ (Novosanis, Belgium) and UCM (Urine Collection Medium, UAntwerp, Belgium) has enhanced the analytical detection of HPV DNA in female urine.
- The Xpert® HPV assay (Cepheid, Sunnyvale) has been validated to detect HPV DNA in cervical samples, but no data are currently available regarding HPV DNA detection in urine.
- The aim of this study is to determine if the Xpert HPV assay is compatible with Colli-Pee™ collected, UCM preserved urine.

**Methods:**

- 15 Colli-Pee™ collected, UCM preserved urine samples (14 previously positive with the Optiplex HPV genotyping DiaMex assay) were analysed - samples originated from a cohort of women participating in a therapeutic HPV vaccination trial and were also characterised by an in-house HPV type specific (TS) qPCR method (UAntwerp, Belgium).
- The participants collected the first void urine self sample at home and sent the collection vial uncooled by mail to the University of Antwerp.

Figure 1. Sample Collection and Workflow




**Results:**

- 14/15 samples gave a valid sample adequacy control (Table 1)
- High positive correlation between CT values obtained by our in-house human DNA (GAPDH) PCR and the Cepheid SAC CT values (Figure 2)
- Very high positive correlation for the CT values for HPV 16 DNA (Figure 3)
- Good agreement between the Cepheid assay, the Luminex based DiaMex assay as well as with our in-house HPV 16/18 TS qPCR.

**Conclusions:**

- These results are very encouraging to further investigate the performance of first void collected, UCM preserved urine in combination with Xpert HPV.
- As both the collection and the detection system can function outside the cold chain, this may lead to innovative HPV testing opportunities in low-resource and point-of-care settings.

**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

Table 1: Raw data of three different detection methods. (nt: not tested)

| | Cepheid GeneXpert | | | Optiplex HPV genotyping DiaMex | | In house TS qPCR | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sample ID | HPV type | HPV (CT) | SAC (CT) | HPV type | MFI | HPV16 (CT) | HPV16 (co/µl) | HPV18 (CT) | HPV18 (co/µl) | GAPDH (CT) |
| CEP1 | 16 | 27.1 | 29.7 | 16 | 4548 | 23.06 | 238000 | nt | nt | 26.33 |
| CEP2 | 16 | 26.4 | 28.8 | 16; 44; 53 | 4308; 180; 225,5 | 23.56 | 173000 | nt | nt | 25.95 |
| CEP3 | 16 | 38.4 | 26.8 | 16 | 1114 | 35.71 | 73.8 | nt | nt | 25.67 |
| CEP4 | 16 | 36.6 | 33.1 | 16 | 2772 | 36.19 | 54.2 | nt | nt | 30.84 |
| CEP5 | 16 | 35.3 | 29.3 | 16 | 3403 | 31.9 | 858 | nt | nt | 24.26 |
| CEP6 | 16 | 34 | 25.4 | 16 | 3445 | 29.84 | 3180 | nt | nt | 24.46 |
| CEP7 | NEG | NEG | 31.3 | 56 | 198 | nt | nt | 0 | NEG | 28.14 |
| CEP8 | NEG | NEG | 29.4 | 18 | 452 | nt | nt | 35.44 | 120 | 28.99 |
| CEP9 | 31,35,33,52,58; 39,68,56,66 | 34.7; 35.0 | 29.7 | 16; 33; 51 | 1638; 385; 95 | 35.22 | 102 | nt | nt | 26.12 |
| CEP10 | ERROR | ERROR | ERROR | 33; 51 | 36; 798 | 0 | NEG | 0 | NEG | 26.66 |
| CEP11 | NEG | NEG | 31.1 | 51; 52 | 64; 469 | nt | nt | 0 | NEG | 27.62 |
| CEP12 | 51, 59 | 32.4 | 28.7 | 59 | 1357 | nt | nt | 0 | NEG | 23.99 |
| CEP13 | 16; 18, 45 | 34.0; 32.3 | 27.9 | 16; 18 | 2975,5 ; 493 | 33.55 | 298 | 34.37 | 239 | 27.55 |
| CEP14 | NEG | NEG | 26.9 | 16 | 995.5 | 35.3 | 96.4 | nt | nt | 22.6 |
| CEP15 | NEG | NEG | 29.3 | NEG | NEG | 0 | NEG | nt | nt | 27.27 |

nt: not tested; CT: cycle threshold; NEG: negative; SAC: sample adequacy control; GAPDH: glyceraldehyde-3-phosphate dehydrogenase, human gene; MFI: Median Fluorescence Intensity; Co: copies; TS: type specific; qPCR: quantitative polymerase chain reaction.

Figure 2. Correlation CT values for human DNA

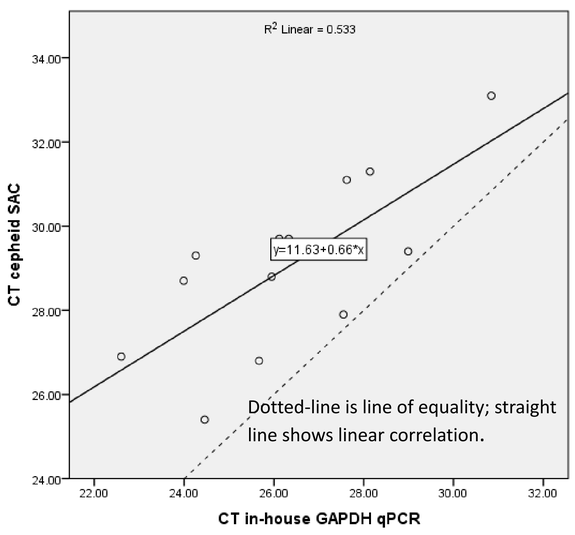



Figure 3. Correlation CT values for HPV16

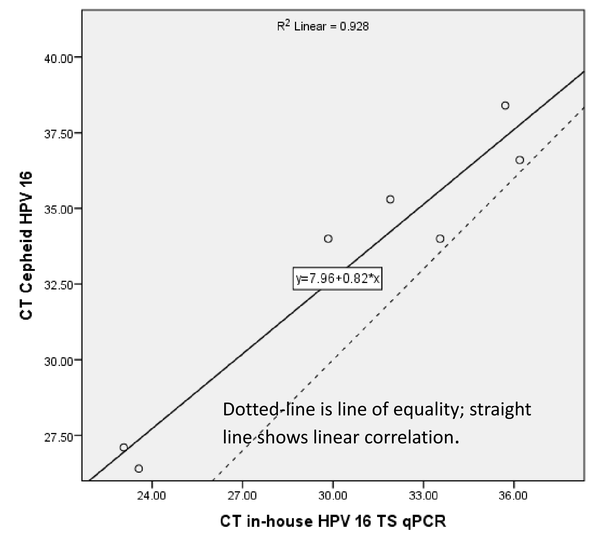



Table 2: Agreement between Cepheid, DiaMex and in-house TS qPCR.

| Type HPV DNA // Alternative assay | Cepheid+ Alternative+ | Cepheid+ Alternative- | Cepheid- Alternative+ | Cepheid- Alternative- | Kappa (CI95%) |
|---|---|---|---|---|---|
| All HPV DNA // DiaMex HPV | 9 | 0 | 4 | 1 | 0.243 (-0.167 – 0.653) not significant |
| HPV16/18 // in-house TS qPCR | 7 | 0 | 3 | 4 | 0.571 (0.183 – 0.959) p = 0.018 |

**Discussion:**

- A linear correlation between the Cepheid CT values and the in-house qPCR CT values was observed
- The 4 discrepant results, Cepheid negative and DiaMex HPV assay positive observed for samples CEP7, CEP8, CEP11 and CEP14 report MIF values less than 1000. The three discrepant results for HPV 16 or 18 between Cepheid and our in-house qPCR were obtained for samples CEP8, CEP9 and CEP14. The copy numbers of HPV 16 or 18 determined by the in-house qPCR for these samples were 120 (HPV18), 102 (HPV16) and 96.4 (HPV16), respectively. So also for these samples, discrepancy may be caused by presence of a limited amount of HPV DNA.
- Using a larger sample volume could have enhanced the HPV detection by the in-house TS qPCR and by the DiaMex assay. Furthermore, for sample CEP9, the two other HPV genotypes present were detected by the GeneXpert.
- Our in-house developed methods have been designed to provide maximal analytical sensitivity. Therefore, the slightly lower analytical sensitivity observed with the Cepheid assay may not be of relevance in a clinical setting.

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**Disclosure:**

Novosanis is a spin-off company of the University of Antwerp.
VA, VKV, BK, and VDP are co-founders and board members of Novosanis.