Preliminary evaluation of the High+Low PapillomaStrip assay with Colli-Pee® collected UCM preserved urine.

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Introduction and objectives:

- HPV testing in first-void urine has been proposed for monitoring impact of HPV vaccination, follow-up of treatment and/or reaching women not participating in cervical cancer screening programmes.
- Use of first-void urine collection device Colli-Pee® (Novosanis, Belgium) and UCM (Urine Conservation Medium, UAntwerp, Belgium) can enhance the analytical detection of HPV DNA in female urine[1,2].
- The High+Low PapillomaStrip test (Operon, S.A.) allows qualitative detection of 37 human Papillomavirus types in DNA samples from cervical smears or biopsies.
- **The aim of this pilot study is to determine whether the PapillomaStrip assay is compatible with self-collected UCM preserved first-void urine samples.**

Methods:

- Twenty-two self-reported HPV positive women collected first-void urine sample, on two subsequent days.
- Samples were collected at home using a Colli-Pee® (Novosanis) urine collection device. The collection tubes were prefilled with UCM preservative and sent by mail to the Antwerp University.
- Sample collection and processing is shown in Figure 1; prior to the PCR tests, 4ml of urine/UCM mixture was concentrated on an ultrafiltration membrane and extracted with easyMag® (bioMérieux).
- Subsequently, the DNA extract was analysed using the Riatol qPCR HPV genotyping assay (AML) and the High+Low PapillomaStrip assay.
- To provide sufficient volume for the Riatol qPCR assay the DNA extract was diluted (35µl DNA extract with 40µl elution buffer).

Results:

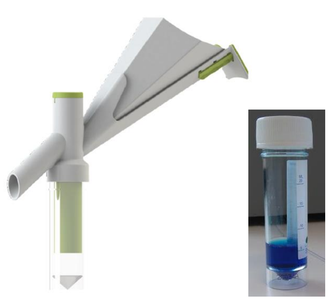
- We observed a 100% agreement between the PapillomaStrip assay outcomes of the first and second day when expressed as hrHPV positive or negative. Five women were negative and 17 were positive for both samples. Also at the level of detected genotypes a very good agreement was observed. When comparing the hrHPV results generated by the Riatol qPCR and the PapillomaStrip assay a kappa coefficient of 0.82 (CI 95%: 0.64–1) was observed. 31 and 10 samples gave respectively concordant positive and negative results. Three samples were negative for Riatol and positive for the PapillomaStrip. These three samples also yielded a positive outcome using other commercial HPV assays. (See Table 1)

Table 1: Overview of the detected genotypes

ID	AML HPVtype pos	Operon HPV
1	16	16
1	16	16
2	59, 67	59, 73, 44, 54, 67, 84
2	67	59, 73, 44, 54, 67, 84
3	18, 31	18, 31, 54
3	18, 31	18, 31, 40, 54, 61
4	58	58, 68, 70
4	58	58, 68, 70
5	16, 31, 53	16, 31, 51, 53, 84
5	16, 31, 53	16, 31, 51, 53, 84
6	neg	68
6	neg	51, 52, 68, 6, 84
7	31, 33	31, 33
7	33	33
8	neg	neg
8	neg	neg
9	35, 53, 67	35, 53, 62, 67
9	35, 53, 67	35, 53, 62, 67
10	16, 33	16, 33, 58, 42
10	16, 33	16, 33, 58
11	neg	35, 45, 62, 70
11	35	35, 45
12	31	31, 43
12	31	31, 43, 54
13	66	66, 70
13	56, 66	66, 70
14	52	52, 44, 54, 70, 74, 81
14	52	52, 44, 54, 70, 74, 81
15	neg	neg
15	neg	neg
16	35	35, 53, 82, 40, 42
16	35	35, 53, 82, 40, 42
17	58	58, 62, 83, 84
17	58	39, 58, 84
18	33	33, 66, 61
18	33	33, 66, 61
19	neg	neg
19	neg	neg
20	neg	neg
20	neg	neg
21	neg	neg
21	neg	neg
22	16, 39, 67	16, 35, 39, 40, 67, 84
22	16, 39, 67	16, 39, 40, 67, 84

Underlined genotypes are not included in both assays. Genotypes shown in blue gave weak signal (< 100, according to the automatic reader)

Figure 1: Samples Collection and workflow



***Colli-Pee®**: Allows capturing fixed volume of guaranteed first-void urine and immediate mixing with preservative buffer.*

*Manual **Amicon filtration[1]**: 4 ml loaded on filter*

NucliSENS® easyMag® extraction. DNA eluted in 55 µl elution buffer

Riatol qPCR HPV genotyping assay at AML (1µl per PCR reaction). 35 µl DNA extract is diluted with 40 µl elution buffer.

High+Low PapillomaStrip assay (Operon, S.A., Zaragoza). 5 µl per PCR reaction.

Conclusions:

- These preliminary results confirm that the High+Low PapillomaStrip test is compatible with self-collected UCM preserved first-void urine.
- Confirmation of performance by testing larger series of first-void samples in a clinical setting is warranted.

References:

1) *A. Vorsters et al. Optimization of HPV DNA detection in urine by improving collection, storage, and extraction. Eur J Clin Microbiol Infect Dis. 2014*
2) *J. Pattyn et al. Human papillomavirus detection in urine: Effect of a first-void urine collection device and time of collection. Journal of Virological Methods*. 2019

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Disclosure:
Novosanis is a spin-off company of the University of Antwerp.
VA, VV, BK, and VDP are co-founders and board members of Novosanis.
R. De Luis and S. Gamen both work at (Operon, S.A.)