Post-collection stability of human papillomavirus DNA in first-void urine

Laeremans M.[1], Mahieu L.[2], De Smet A.[2], Pasmans D.[1], Cos P.[2], Beyers K.[1], **Vankerckhoven V.**[1], Vorsters A.[2]

1 Novosanis, Belgium | 2 University of Antwerp, Belgium

BACKGROUND & OBJECTIVES

Women have shown to accept and prefer urine self-sampling for HPV-based cervical cancer screening. Colli-Pee® allows for user-friendly, standardized and volumetric collection of first-void urine. The collector tube is prefilled with UCM, a proprietary preservative. Devices can be shipped via postal mail to the laboratory which overcomes the most common reasons for non-attendance of screening visits. The aim of the study was to assess the effect of environmental conditions on the degradation of urinary analytes such as HPV DNA.

METHODS

Five urine samples were collected with Colli-Pee® to evaluate the stability of HPV DNA in UCM-preserved urine samples. UCM was spiked with HPV16 DNA to obtain a concentration of 100 DNA copies/µL in the final aliquot. Afterwards, urine was added in a 2:1 (urine:UCM) ratio to mimic real-life use of Colli-Pee® prefilled with UCM. Storage at RT was used as the reference condition since UCM allows for storage at RT for 7 days. DNA extraction was performed using the NucliSENS EasyMAG, and PCR was based on TaqMan technology using the Roche LightCycler 480 Real-Time PCR System. (Depicted in Figure 1)

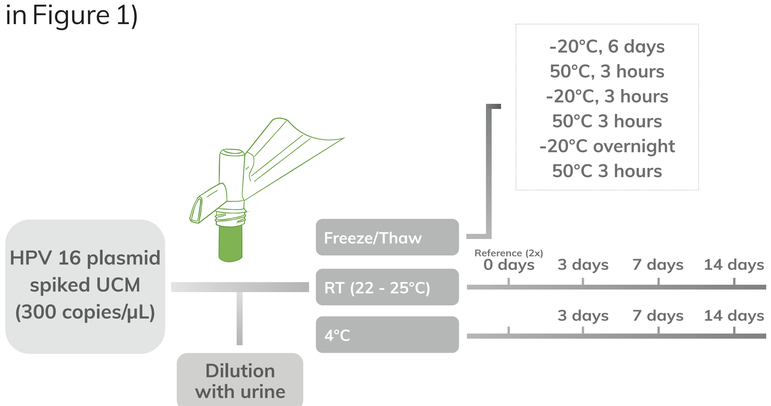

Figure 1: Visual representation of the experimental design.

RESULTS

A total of 45 aliquots (i.e., 9 for each of 5 subjects) were analyzed, of which 44 were positive for HPV16. One sample was negative for HPV16 after 14 days of storage at RT. The sample of the same subject showed an outlying result after 7 days of storage at RT. In order to analyze the results, Ct values with a negative result for HPV16 were defined as the cut-off values i.e., a cycle threshold of 40. After 3, 7 and 14 days of storage respectively, average Ct-values ±SD were 32.76±0.31, 33.53±1.92 and 34.16±3.27 at RT, and 32.75±0.36, 32.70±0.18, and 33.00±0.32 at 4°C. The slight increase in the average Ct values was mainly due to one sample which showed a higher Ct value after 7 and 14 days. However, the other samples showed no change in Ct-value over time. The average Ct-value was 32.60±0.22 after undergoing F/T cycles (Figure 2).

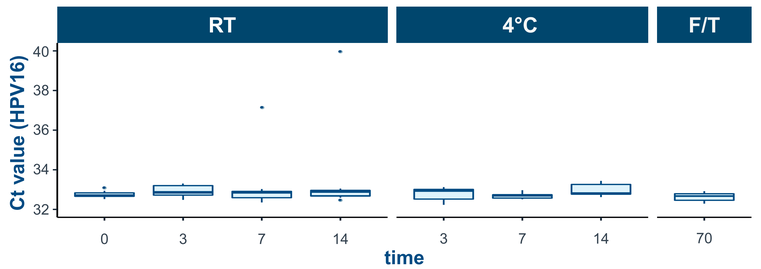

Figure 2: Visual representation of the raw data i.e., Ct values of HPV16 per condition: each temperature i.e., RT, 4°C and F/T, and for each time point i.e., 0d, 3d, 7d and 14d. The data is depicted as Box plots which show a five-number summary: the minimum, the maximum, the median of the samples and the first and third quartiles.

Effect of temperature and time of storage

The cycle threshold for HPV16 detection for different times of storage at different temperatures showed no significant differences (see Table 1).

HPV16-Ct	0 days	3 days	7 days	14 days
RT	Reference i.e., 32.76	0.14 [-0.08, 0.35]; p = 0.8420	0.77 [-0.58, 2.13]; p = 0.2727	1.41 [0.05, 2.76]; p = 0.0507
4°C		-0.01 [-0.22, 0.21]; p = 0.9943	-0.06 [-0.27, 0.16]; p = 0.9371	0.25 [0.03, 0.46]; p = 0.7213

Table 1: Regression analysis to assess the effect of the interaction between time and temperature. Mixed effect linear models were used to account for repeated measurements from one sample. Cycle threshold for HPV16 was used as the outcome, and the combination of time (t = 3d, 7d, 14d) and temperature (T = RT, 4°C) was used as the independent variable compared with a fresh sample as reference (t = 0, T = RT). The reference value represents the intercept of the regression model. The 95% confidence interval is shown between brackets, followed by the p-value. * represents a significant difference from the reference i.e., $p < 0.05$.

Effect of freeze/thaw cycles

The outcome Ct values for HPV16 detection of samples exposed to F/T cycles were similar to the reference samples, as no significant differences were observed. Exposure of samples to F/T cycles had no effect on sample preservation when UCM was used as a preservative.

HPV16-Ct	RT	FT
0d	Reference i.e., 32.76	-0.16 [-0.32, 0.00]; p = 0.079
7d	Reference i.e., 32.72	-0.13 [-0.42, 0.16]; p = 0.311

Table 2: Regression analysis to assess the effect of freeze/thaw cycles. Mixed effect linear models were used to account for repeated measurements from one sample. Cycle threshold for HPV16 was used as the outcome, and freeze/thaw condition was used as the independent variable compared with a fresh reference (t = 0d, RT). The reference value represents the intercept of the regression model. The 95% confidence interval is shown between brackets, followed by the p-value. * represents a significant difference from the reference i.e., $p < 0.05$.

CONCLUSION

Spiked HPV16 DNA stability in UCM-preserved urine samples was shown in post-collection conditions i.e., F/T cycles, and storage at 4°C and RT. Samples of one subject showed outlying results after storage at room temperature after 7 and 14 days. This indicates the added value of an internal control to provide feedback on sample quality from collection to analysis. Our results show that home-based collection and postal shipment of first-void urine samples for HPV-based cervical cancer screening is feasible when UCM is used as a preservative. Time of storage and temperature had no effect on sample preservation when UCM was used as a preservative.

EUROGIN Virtual, May 2021

Presenting author: vanessa.vankerckhoven@novosanis.com

novosanis

University of Antwerp