Determination of the optimal first-void urine collection volume for the detection of viral and host biomarkers, and evaluation of an internal control. *CASUS study*

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OBJECTIVES

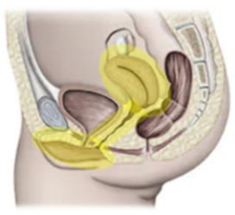
The goal of the CASUS project is to develop the first fully molecular integrated cervical cancer screening approach, based on first-void (FV) urine as an easily accessible and non-invasive source of biomarkers.
In this study (CASUS WP1) we have;

1) Investigated whether the **FV urine collection volume** has an effect on detection of human and viral DNA endpoints (i.e. GAPDH, ACTB, β-globin, and HPV) by comparing three different FV urine collection volumes using the Colli-Pee® FV5000 (20 ml) and Colli-Pee Small Volumes (10 and 4 ml) devices

2) Evaluated different **DNA extraction protocols**

3) Introduced a universal non-human **internal control** (IC DNA) in the urine conservation medium (UCM)

RATIONALE



First-void urine
(initial stream of urine)

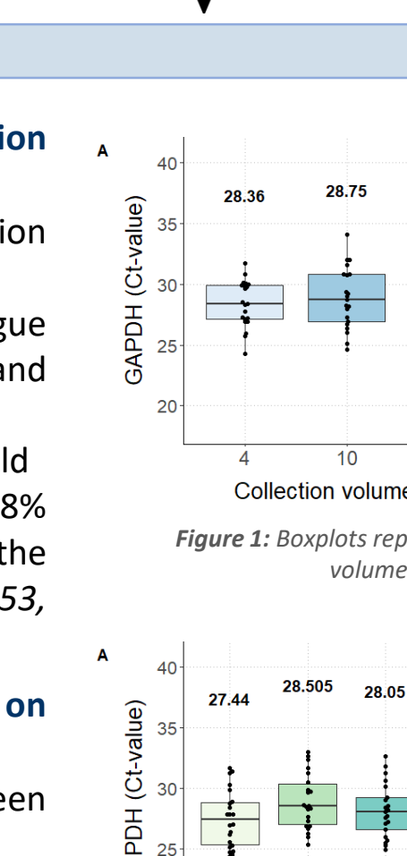
Captures impurities lining the urethra opening including transudated antibodies and biomarker-containing mucus and debris from exfoliated cells originating from female genital organs.

Non-invasive | Home-based | Likely preferred | Reach screening non-participants

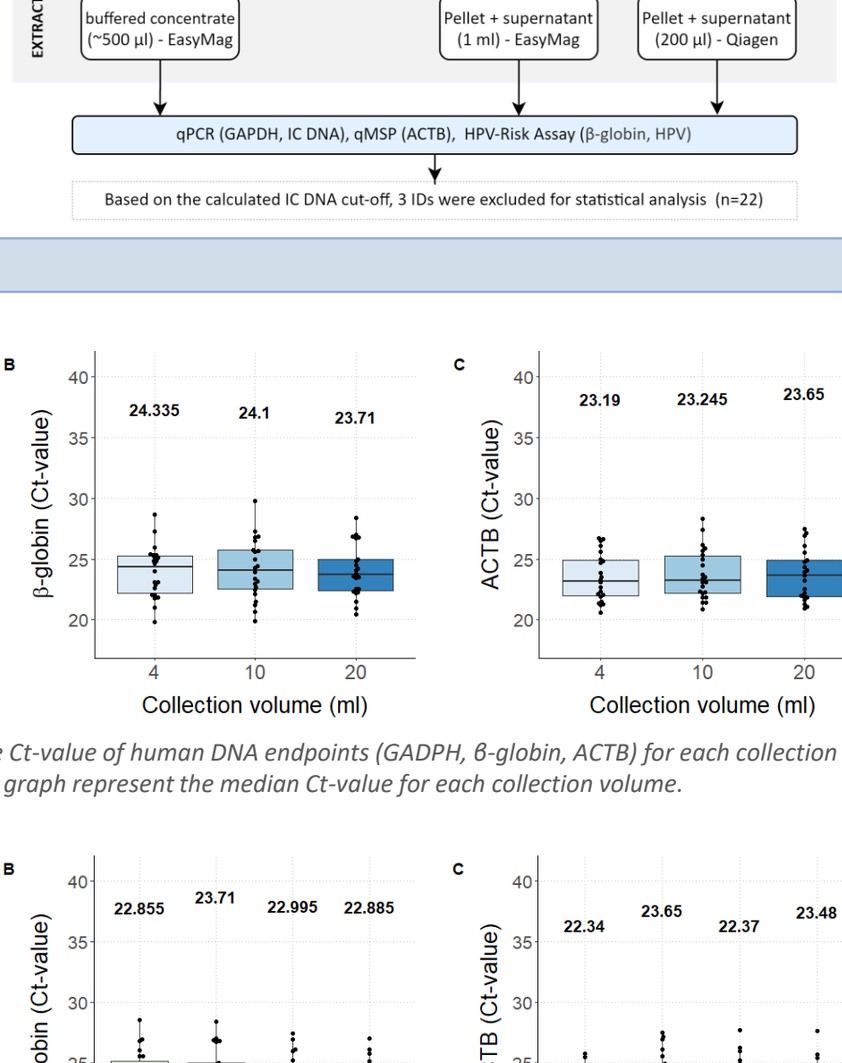
METHODS

Inclusion criteria

- Women age 18 years or older
- Self-reported high-risk (hr)HPV infection in last 6 months



Study design



RESULTS

1) Comparison between three different FV urine collection volumes using DNA extraction method 2 (Fig. 1)

- No significant differences between the three different collection volumes for human DNA endpoints
- Highly significant ($p < 0.0001$) Pearson correlations and analogue slopes of the correlation curves were found between GAPDH and ACTB, for all collection volumes
- Order of collection did not have a significant effect on DNA yield
- FV urine samples collected with the 4 ml device showed 31.8% (7/22) Hr-HPV positivity, the 10 ml device 45.4% (10/22) and the 20 ml device 36.3% (8/22) *(Cohen's Kappa values: $K_{4/10} = 0.53$, $K_{4/20} = 0.90$, $K_{10/20} = 0.63$)*

Figure 1: *Boxplots representing the Ct-value of human DNA endpoints (GADPH, ß-globin, ACTB) for each collection volume. Numbers in graph represent the median Ct-value for each collection volume.*

2) Comparison between different DNA extraction methods on 20 ml collection volume (Fig. 2)

- Variation in results and significant differences between extraction methods for human DNA endpoints
- EM2 gave lowest yield overall and EM1 highest
- DNA extracted using EM1 showed 45.4% (10/22) Hr-HPV positivity, EM2 36.3% (8/22), EM3 40.9% (9/22), and with EM4 36.3% (8/22) *(Fleiss' Kappa value: K = 0.86)*

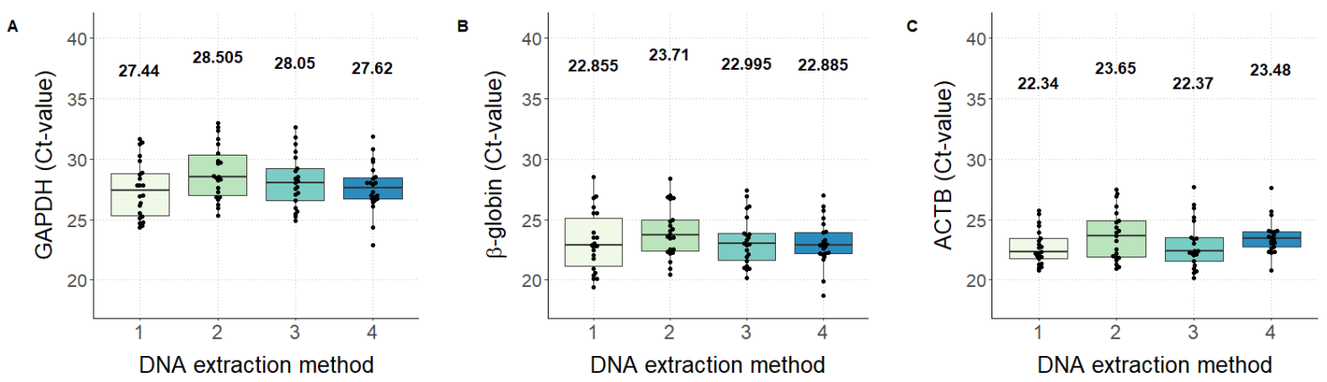


Figure 2: *Boxplots representing the Ct-value of human DNA endpoints (GADPH, ß-globin, ACTB) for each DNA extraction methods. Numbers in graph represent the median Ct-value for each extraction method.*

3) Potential of an internal quality control

- Median IC DNA yields are not significantly different between collection volumes
- Different yield between DNA extraction methods which are directly linked to the lower implemented volume for extraction. Lowest IC DNA yield for EM4 since IC DNA is cell free DNA and thus not concentrated by the pellet
- Cut-off calculated using the mean + 2*Std Dev and used for exclusion of three samples (~Ct-value > 37)
- A linear model showed a significant effect of time between collection and storage on IC DNA values ($p = 0.01$). No effect of time between collection and storage was seen on all human DNA endpoints (i.e. GAPDH, β-globin, ACTB)

CONCLUSION

This data from CASUS WP1 lead to the selection of the optimal Colli-Pee collector device and DNA extraction method that will be used for validation of a fully molecular integrated cervical cancer screening approach based on primary HPV DNA testing and host cell methylation marker triage.

Collection volumes	▪ No differences between collection volumes for human DNA endpoints ▪ 4 and 10 mL collection advantageous for high-throughput testing
extraction methods	▪ Extraction method has an effect on DNA yield, EM1 provided best results ▪ Extraction method 3 is most advantageous for high-throughput testing
Internal control	▪ Internal control shows potential to be used in further research ▪ Final cut-off will be calculated based on a larger validation trial (CASUS project)

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Disclosure *– Novosanis is a spin-off company of the University of Antwerp. AV is co-founder and former board member of Novosanis. RDMS has a minority stake in Self-screen B.V. (The Netherlands), a spin-off company of VU University Medical Center Amsterdam, which owns patents related to this work. Other authors declare that they have no conflict of interest.*

CASUS collaborating partners: