Comparison of a VLP-based and GST-L1-based multiplex immunoassay to detect vaccine-induced HPV-specific antibodies in first-void urine

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Objectives

Vaccine-induced human papillomavirus **(HPV) antibodies (Abs)** originating from cervicovaginal secretions (CVS) were recently shown to be **detectable in first-void (FV) urine** *(Van Keer et al. 2019).* This presents a novel opportunity for non-invasive sampling to monitor HPV antibody status in women participating in large epidemiological studies and HPV vaccine trials. With a view towards method optimization, this study compared measurement of **HPV antibodies in FV urine** using a **multiplex L1/L2 virus-like particles (VLP)-based ELISA** (M4ELISA) with previously reported results using a **glutathione S-transferase (GST)-L1-based immunoassay** (GST-L1-MIA).

Methods

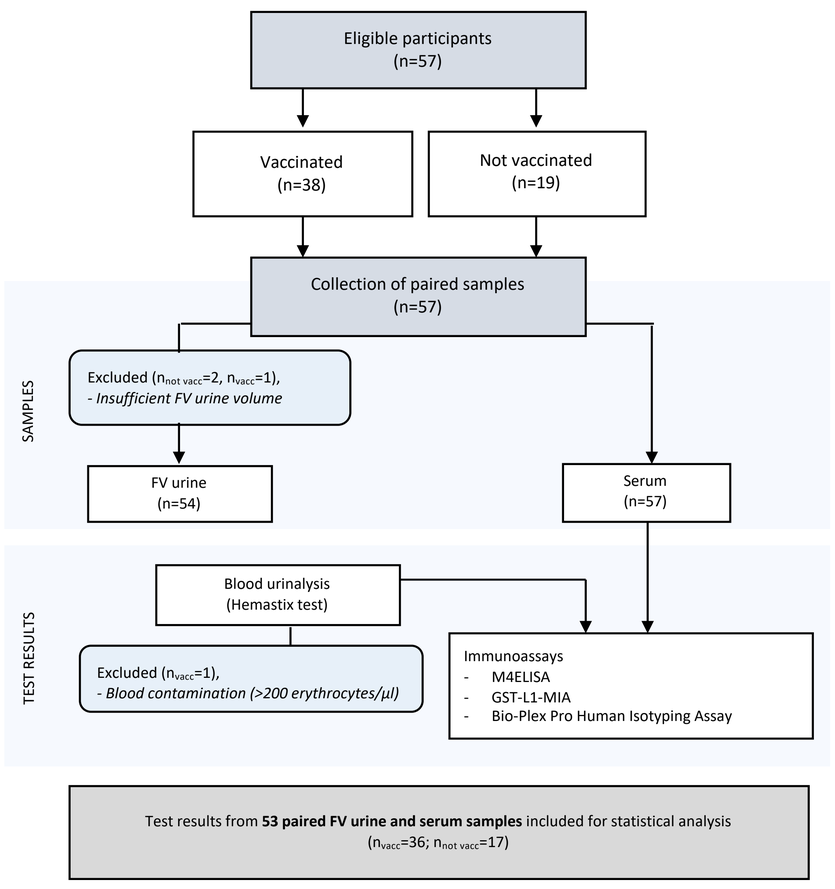
Study design

We tested **FV urine and serum samples** from 19-to 26-year-old healthy women, *unvaccinated* (n=17) or *vaccinated* with either the bi- or quadrivalent HPV-vaccine during adolescence (n=36)

HPV6/11/16/18 antibodies were measured using M4ELISA and compared with GST-L1-MIA results. **Inter-assay and inter-specimen correlations were examined** using the *Spearman's rank test (r_s)*

Trial registration ID: NCT02714114

Flow diagram of the study:



Sample collection

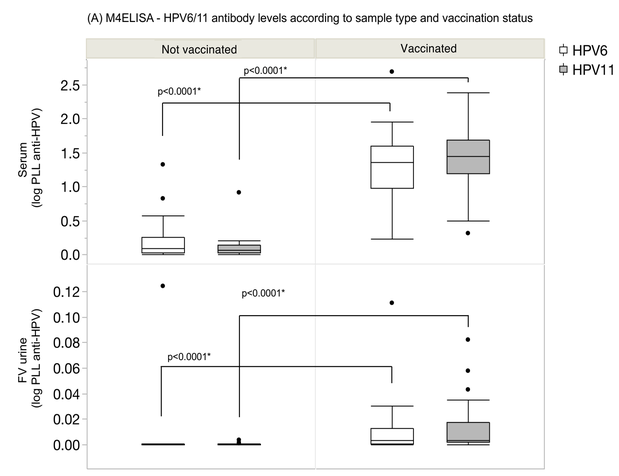
- **FV urine** was collected with the *Colli-Pee® device* (Novosanis, Belgium) containing urine conservation medium (UCM)

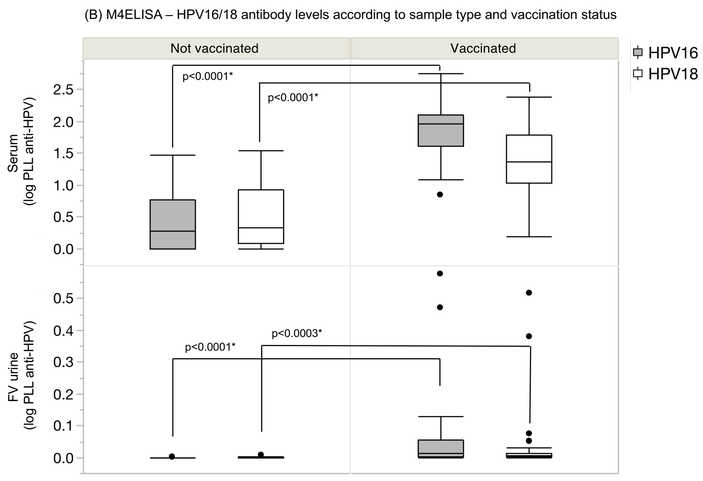
Conclusion

- FV urine HPV antibody **detection is comparable with both assays**, further supporting this non-invasive sampling method as an option for HPV vaccine assessment
- **Approaches to improve the sensitivity** and **larger studies** are warranted to determine the feasibility of FV urine for HPV antibody detection

Results

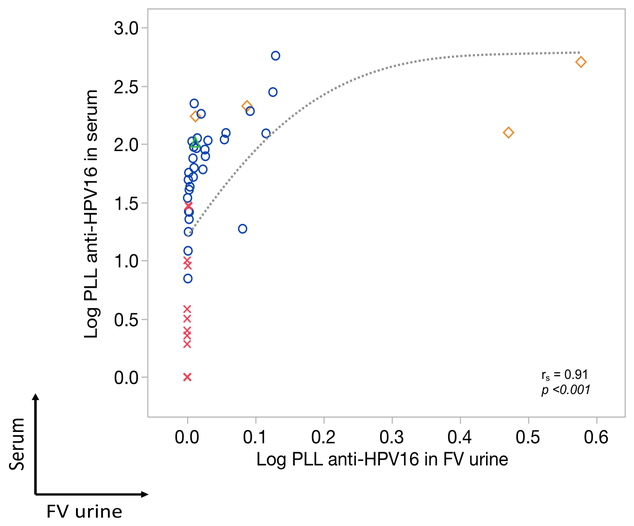
1/ M4ELISA measured HPV antibody levels





- **Vaccinated** women had **significantly higher** HPV6/11/16/18 **antibody levels** in both FV urine and serum compared with those unvaccinated (FV urine p=0.0003; serum p ≤ 0.0001). *P-values indicated by * indicate a significant difference in median Ab levels between vaccinated and not vaccinated women (Mann-Whitney U test).*

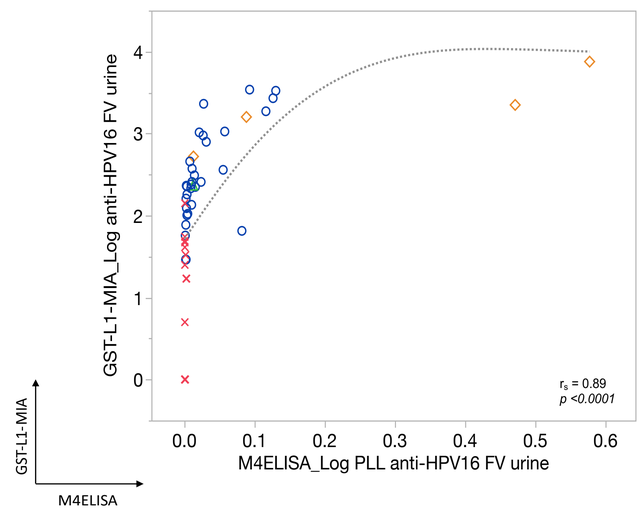
2/ M4ELISA comparison between FV urine and serum

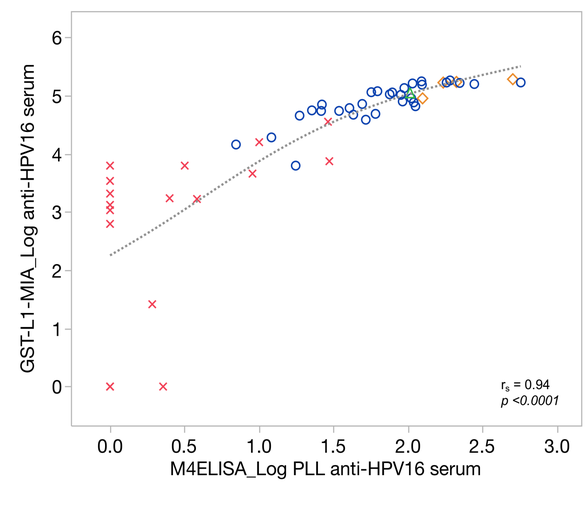


- HPV antibody levels in FV urine and serum showed a **significant positive correlation** (M4ELISA anti-HPV6/11/16/18, r_s = 0.85/0.86/0.91/0.79, p ≤ 0.001)
- A sensitivity analysis that excluded high values was performed to determine their influence on the Spearman's correlations. The analyses showed no differences in correlations

4vHPV vaccine
2vHPV vaccine
2v- and 4vHPV vaccine
Unvaccinated

3/ Comparison between M4ELISA and GST-L1-MIA





- Despite assay differences, there was **good agreement between M4ELISA and GST-L1-MIA** (FV urine anti-HPV6/11/16/18, r_s = 0.86/0.83/0.89/0.53, p ≤ 0.0001; serum anti-HPV6/11/16/18, r_s = 0.93/0.89/0.94/0.75, p ≤ 0.0001)
- A sensitivity analysis that excluded high FV urine values was performed to determine their influence on the Spearman's correlations. The analyses showed no differences in correlations
- **Expected better sensitivity** for **M4ELISA** was **not observed**

Reference – (1) *Pattyn, J. et al. A. Comparison of a VLP-based and GST-L1-based multiplex immunoassay to detect vaccine-induced HPV-specific antibodies in first-void urine. Ready for submission. (2) Van Keer, S. et al. First-void urine as a non-invasive liquid biopsy source to detect vaccine-induced human papillomavirus antibodies originating from cervicovaginal secretions. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology 117, 11-18. (QR Code)* **Aknowledgement** - *This work is supported by the industrial Research Fund of the University of Antwerp. J. Pattyn is supported by a PhD fellowship from the Royal Belgian Academy of Medicine (Grant GSK Vaccines).*

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