



University of Antwerp

Centre for the Evaluation of Vaccination
Vaccine and Infectious Disease Institute

The standing of self-collected urine samples for DNA methylation in cervical screening

Severien Van Keer

HPV Prevention and Control Board Meeting

Antwerp, Belgium – Friday, June 3rd, 2022

Why first-void urine?

- Collects cervicovaginal secretions non-invasively¹
- Well-accepted^{2,3}
- Good HPV DNA agreement between first-void urine and cervical samples⁴
- Similar clinical accuracy (CIN2+; <CIN2) of hrHPV-DNA based PCR testing in (first-void) urine compared to clinician-collected cervical samples^{5,6}



Open access Original research

BMJ Open Clinical performance and acceptability of self-collected vaginal and urine samples compared with clinician-taken cervical samples for HPV testing among women referred for colposcopy. A cross-sectional study

Dorthe Ørnskov ¹, Kirsten Jochumsen, ² Pernille Husted Steiner, ³ Ivan Moulun Grunnet, ² Annemette Wildfang Lykkebo, ³ Marianne Waldstrøm ¹

Journal of Clinical Virology 107 (2018) 52–56

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

VALHUDES: A protocol for validation of human papillomavirus assays and collection devices for HPV testing on self-samples and urine samples

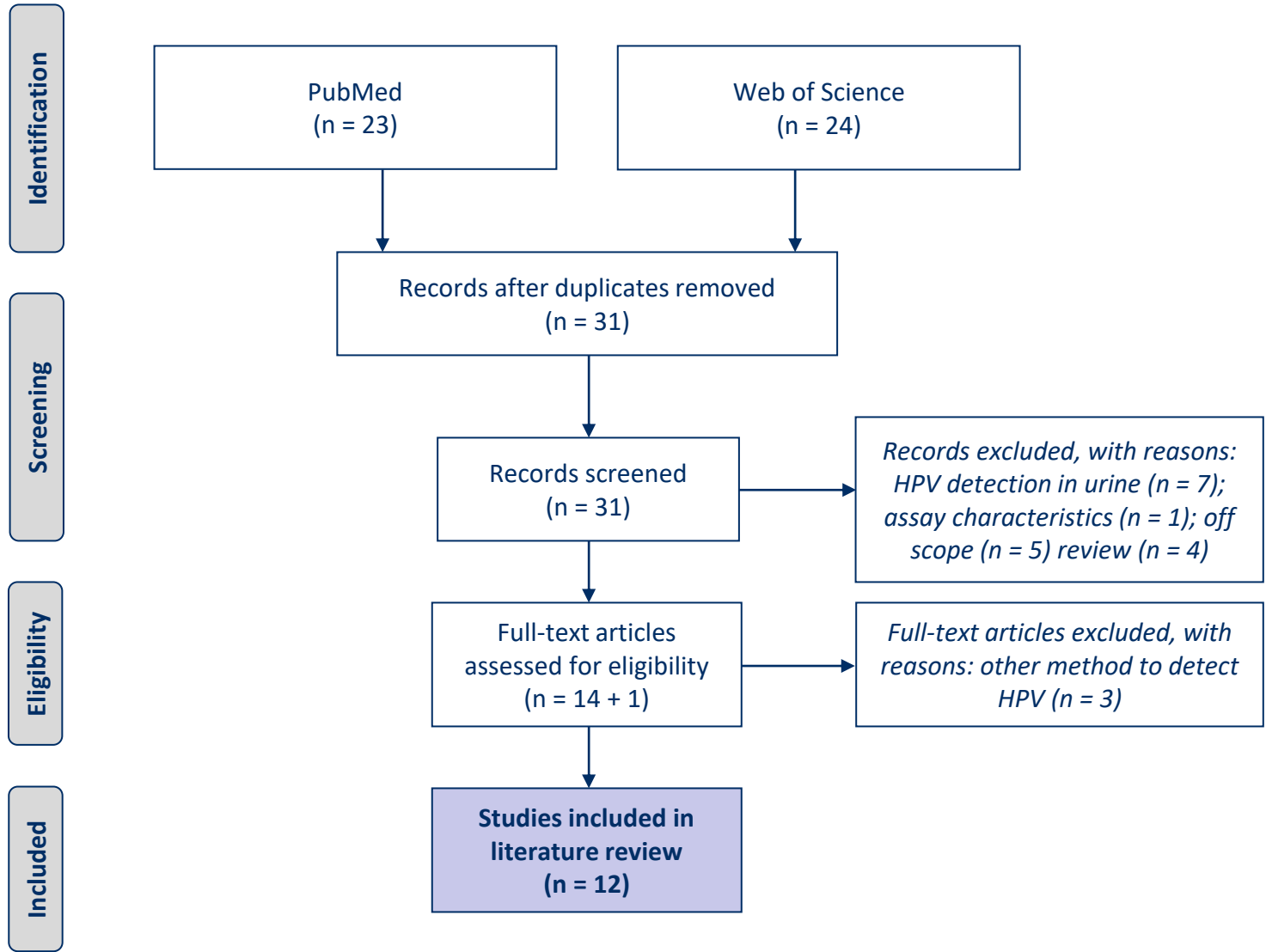
M. Arbyn^{a,*}, E. Peeters^a, I. Benoy^{b,c,d}, D. Vanden Broeck^{b,c,d,e}, J. Bogers^{b,c,d,e}, P. De Sutter^f, G. Donders^{g,h,i}, W. Tjalma^{j,k}, S. Weyers^l, K. Cuschieri^m, M. Poljakⁿ, J. Bonde^o, C. Cocuzza^p, F.H. Zhao^q, S. Van Keer^r, A. Vorsters^r

¹Vorsters A et al. (2012) Eur J Clin Microbiol Infect Dis; ²Leeman A et al. (2017) BJOG; ³Rohner E et al. (2020) J. Women’s Health; ⁴Pathak N et al. (2014) BMJ; ⁵Ørnskov D et al. (2021), BMJ Open; ⁶Van Keer S et al. (2021) Gynecol Oncol

Review of the literature

(June 1st, 2022)

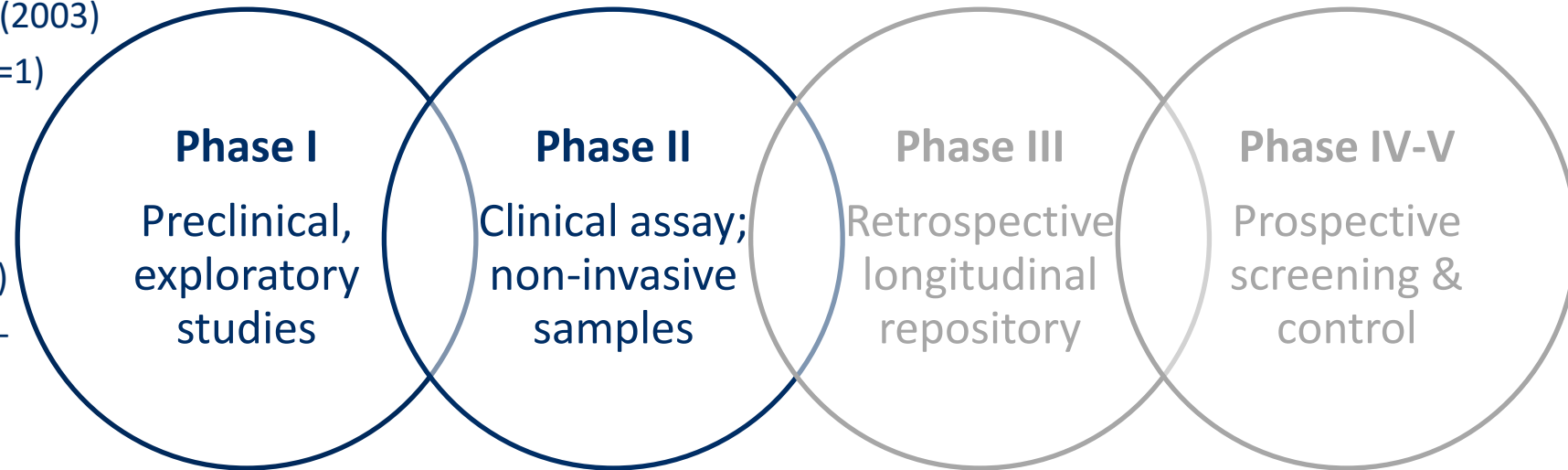
- PubMed ('All Fields Contain', English, Human)
- Web of Science ('Title/Keywords/Abstracts')
- Search terms: marker* OR biomarker*; AND HPV*, human papillomavirus*, human papilloma virus*, cervical cancer(s), cervical carcinoma*, OR cancer(s) of the cervix; AND urine



Updated from Van Keer S, et al. (2017) Eur J Obstet Gynecol Reprod Bio

Results according to phase of biomarker development

- **Enzyme activity** (n=1)
 - Lee SH et al. (2003)
- **Protein-based** (n=1)
 - Garbett NC et al. (2014)
- **Volatile organic compounds** (n=1)
 - Diaz de León-Martinez L et al. (2021)



- **Microscopy-based** (n=1)
 - Sundararajan SK et al. (2017)
- **MiRNA-based** (n=1)
 - Aftab M et al. (2021)

Etzioni R et al. (2003) The case for early detection

- **Host and viral gene methylation** (n=1)
 - Guerrero-Preston R et al. (2016)
- **Host gene methylation** (n=6)
 - Feng QH et al. (2007); Hoffstetter R et al. (2017); Snoek BC et al. (2019); van den Helder R et al. (2020 & 2022); Van Keer S et al. (2021)

Growing evidence on the clinical performance of host and viral DNA methylation in urine for cervical cancer screening

Research Article

Cancer Prevention Research

Molecular Triage of Premalignant Lesions in Liquid-Based Cervical Cytology and Circulating Cell-Free DNA from Urine, Using a Panel of Methylated Human Papilloma Virus and Host Genes



Rafael Guerrero-Preston^{1,2}, Blanca L. Valle¹, Anne Jedlicka³, Nitesh Turaga¹, Oluwasina Folawiyajo¹, Francesca Pirini¹, Fahcina Lawson¹, Angelo Vergura¹, Maartje Noordhuis^{1,4}, Amanda Dziedzic¹, Gabriela Pérez¹, Marisa Renehan¹, Carolina Guerrero-Diaz¹, Edgar De Jesus Rodriguez², Teresa Diaz-Montes², José Rodríguez Orengo⁵, Keimari Méndez², Josefina Romaguera², Bruce J. Trock⁷, Liliانا Florea⁸, and David Sidransky¹

Papillomavirus Research 9 (2020) 100193

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Papillomavirus Research

journal homepage: www.elsevier.com/locate/pvr



Methylation analysis in urine fractions for optimal CIN3 and cervical cancer detection

Rianne van den Helder^{a,b}, Nienke E. van Trommel^b, Annina P. van Splunter^a, Birgit I. Lissenberg-Witte^c, Maaïke C.G. Bleeker^d, Renske D.M. Steenbergen^{b,e}

^aAmsterdam UMC, Vrije Universiteit Amsterdam, Departments of Pathology, Cancer Center Amsterdam, Amsterdam, the Netherlands
^bAmsterdam UMC, Vrije Universiteit Amsterdam, Departments of Gynecologic Oncology, Cancer Center Amsterdam, Amsterdam, the Netherlands
^cAmsterdam UMC, Vrije Universiteit Amsterdam, Departments of Epidemiology and Biostatistics, Amsterdam, the Netherlands

SCIENTIFIC REPORTS

OPEN Cervical cancer detection by DNA methylation analysis in urine

Barbara C. Snoek¹, Annina P. van Splunter¹, Maaïke C. G. Bleeker¹, Maartje C. van Ruiten¹, Daniëlle A. M. Heideman¹, W. Frederik Rurup², Wina Verlaet¹, Hans Schotman³, Mignon van Gent⁴, Nienke E. van Trommel⁶ & Renske D. M. Steenbergen¹

Received: 4 October 2018

CLINICAL CANCER RESEARCH | PRECISION MEDICINE AND IMAGING

HPV and DNA Methylation Testing in Urine for Cervical Intraepithelial Neoplasia and Cervical Cancer Detection

Rianne van den Helder^{1,2}, Renske D.M. Steenbergen², Annina P. van Splunter², Constantijne H. Mom³, Ming Y. Tjong³, Ivonne Martin⁴, Fleur M.F. Rosier-van Dunné⁵, Irene A.M. van der Avoort⁶, Maaïke C.G. Bleeker², and Nienke E. van Trommel¹

www.nature.com/scientificreports

scientific reports

OPEN Triage of human papillomavirus infected women by methylation analysis in first-void urine

Severien Van Keer^{1,2,3*}, Annina P. van Splunter², Jade Pattyn¹, Annemie De Smet¹, Sereina A. Herzog³, Xaveer Van Ostade⁴, Wiebren A. A. Tjalma^{5,6}, Margareta Ieven⁷, Pierre Van Damme¹, Renske D. M. Steenbergen² & Alex Vorsters¹

Original Article

Evaluation of DNA methylation in promoter regions of *SFRP4* and *ZAR1* in urine and plasma of women with cervical lesions

Rene Hoffstetter^{1,2,3*}, Ismael Riquelme^{1,2*}, Alejandra Andana^{1,2}, Carmen G. Ili^{1,2}, Kurt Buchegger^{1,2}, Hernán Vargas⁴, Priscilla Brebi^{1,2}, Juan Carlos Roa⁵

Clinical performance host and viral gene methylation from literature

Methylation markers	Study population	Sample	AUC	Endpoint	Ref
ZNF516; FKBP6; INTS1; and HPV16-L1 (4-gene biomarker panel)	n=40 hrHPV+	ccfDNA 10ml urine (flash frozen)	0.86	CIN2+ vs. ≤CIN1	Guerrero-Preston R et al. (2016)
SFRP4 and ZAR1 (2-gene biomarker panel)	nSCC=31; nNILM=60 hrHPV	pellet ≥10ml first-void urine (crystal violet)	0.63	SCC vs. NILM	Hoffstetter R et al. (2017)
FAM19A4; GHSR; PHACTR3; PRDM14; SST; and ZIC1 (individual genes)	ncancer=38; ncontrol=47 82% hrHPV+	pellet 15ml urine	0.74-0.89	Cancers vs. healthy controls	Snoek BC et al. (2019)
ASCL1; GHSR; LHX8; SST; and ZIC1 (individual genes)	nCIN3+=47; ncontrol=27 hrHPV	pellet 15ml urine (EDTA)	0.87-0.97 0.62-0.79	Cancers vs. healthy controls CIN3 vs. healthy controls	van den Helder R et al. (2020)
ASCL1; ST6GALNAC5; GHSR; LHX8; SST; and ZIC1 (individual genes)	nCIN2+=19; n≤CIN1=14 hrHPV+	4ml first-void urine (UCM)	0.62-0.80	CIN2+ vs. ≤CIN1	Van Keer S et al. (2021)
ASCL1; GHSR; LHX8; SST; and ZIC1 (individual genes)* ASCL1/LHX8 (bi-marker panel)**	nCIN3+=181; ncontrol=64 87% hrHPV+	pellet 15ml urine (EDTA)	0.72-0.83* 0.84**	CIN3+ vs. healthy controls	van den Helder R et al. (2022)

All studies applied methylation specific qPCR on bisulphite converted DNA.

CIN: cervical intraepithelial neoplasia; SCC: squamous cell carcinoma; NILM: negative for intraepithelial lesion or malignancy.

Developing a complete cervical cancer screening solution based on first-void urine self-sampling – CASUS trial

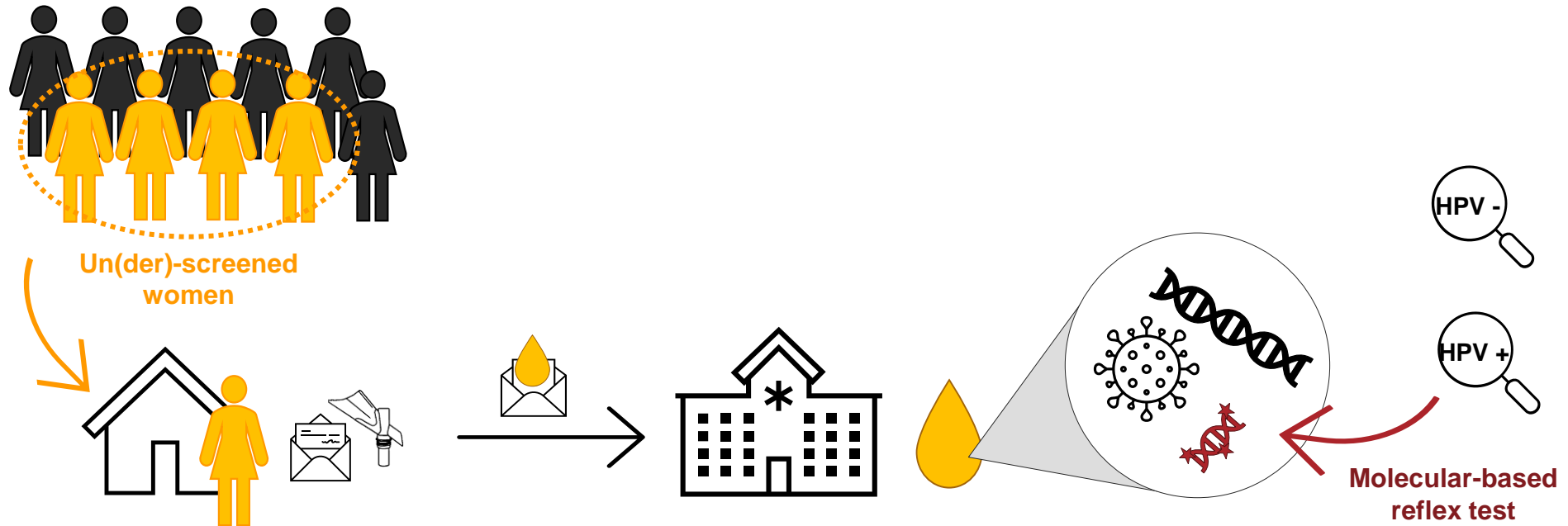
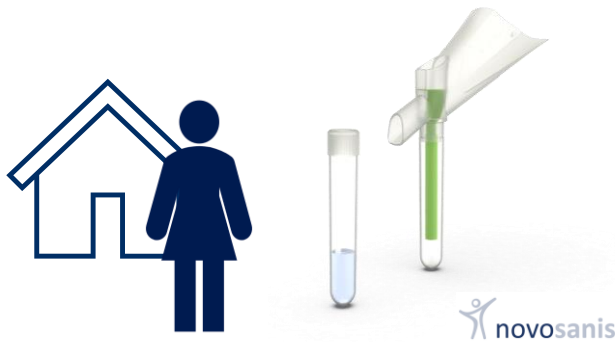




Figure: Van Keer S et al. (2021) HPV World

Developing a complete cervical cancer screening solution based on first-void urine self-sampling – CASUS trial



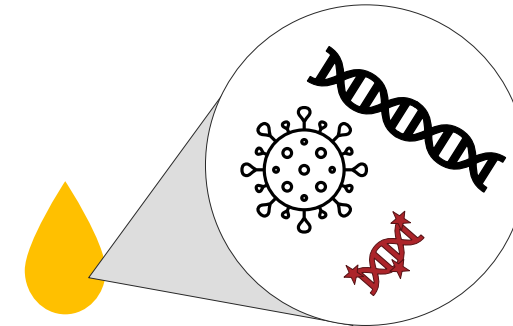
Home-collected first-void urine
(Colli-Pee Small Volumes, with UCM)

Article
Impact of Collection Volume and DNA Extraction Method on the Detection of Biomarkers and HPV DNA in First-Void Urine

Laura Teblick ^{1,*}, Severien Van Keer ¹, Annemie De Smet ¹, Pierre Van Damme ¹, Michelle Laeremans ², Alejandra Rios Cortes ², Koen Beyers ², Vanessa Vankerckhoven ^{1,2}, Veerle Matheussen ^{3,4,5}, Renee Mandersloot ⁶, Arno Floore ⁶, Chris J. L. M. Meijer ^{6,7}, Renske D. M. Steenbergen ⁷ and Alex Vorsters ¹

WP 1



Quantitative methylation-specific PCR (qMSP)

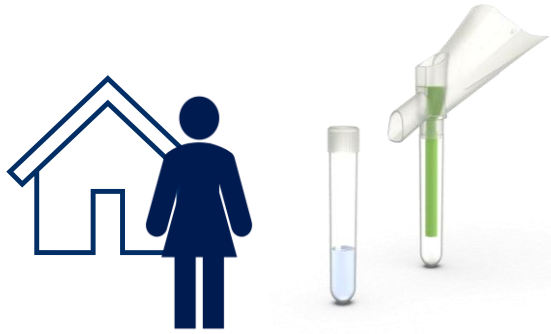


qMSP measuring methylation of host cell genes in bisulphite converted urine DNA

WP 2

WP 3 & 4 – Validate the first-void urine-based complete cervical cancer solution for detection of cervical (pre)cancer

Study design CASUS WP 3 & 4



Home-collected first-void urine
(Colli-Pee Small Volumes, with UCM)

INDEX TEST



Colposcopy/histology/pathology

REFERENCE TEST

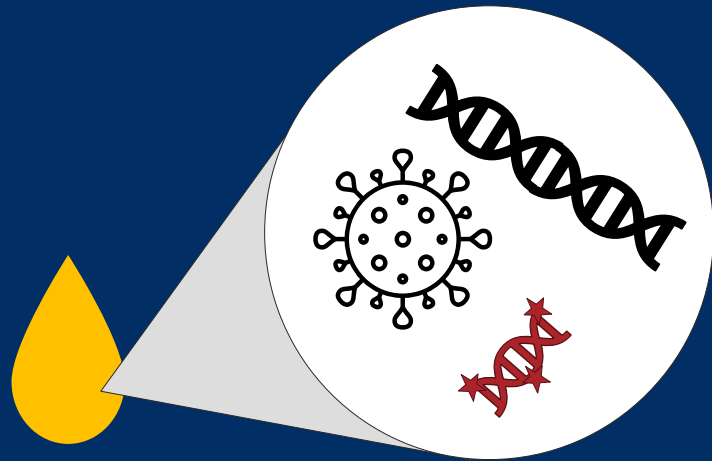
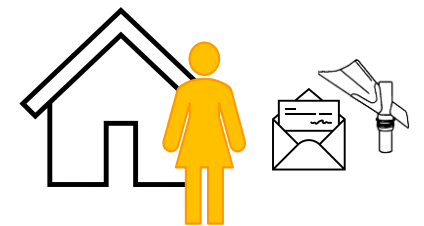
Results CASUS WP 3 & 4

- Unpublished data – cannot be distributed or published yet

A complete cervical cancer screening solution based on first-void urine self-sampling ?

- Similar clinical accuracy of hrHPV-DNA based PCR testing in urine compared to clinician-collected cervical samples
- Host cell methylation marker levels in first-void urine increase with increasing severity of disease
- Methylation of host cell genes discerns <CIN2 from CIN3+ and Cancer in first-void urine

- Collected at home



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