COMPARATIVE ANALYSIS OF HPV GENOTYPING AND MICROBIOME PROFILE IN UTERINE CERVIX SAMPLES OBTAINED BY URINE, SELF-COLLECTION AND HEALTHCARE PROFESSIONALS

Fernanda Dahrouge Chiarot [1,2], Cristina Paula Castanheira[3,4], Noely Paula Cristina Lorenzi[5], Maricy Tacla[6], Adhemar Longatto-Filho[7], Edmund C. Baracat[8], José Maria Soares-Jr[8], Rossana Mendonza López[9,10], Luisa Lina Villa[9,10], Gustavo A. R. Maciel[8], Lara Termini[9,10].

1. Postgraduate Program in Oncology, Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brazil, 2. Departament of Molecular Biology - IPOGLab - Gynecologic Oncology Research Institute, Sao Paulo, Brazil, 3. Department of Obstetrics and Gynecology, Hospital das Clinicas HCFMUSP, Universidade de Sao Paulo, Sao Paulo, Brazil, 4. Department of Obstetrics and Gynecology, Conjunto Hospitalar Mandaqui, Sao Paulo, Brazil, 5. Department of Gynecology, Hospital Universitario da Universidade de Sao Paulo, Sao Paulo, Brazil, 6. Department of Obstetrics and Gynecology, Hospital das Clínicas HC-FMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil, 7. Life and Health Science Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal, 8. Structural and Molecular Gynecology Laboratory (LIM-58), Gynecology Discipline, Department of Obstetrics and Gynecology, Hospital das Clínicas HC-FMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, Brazil, 9. Instituto do Cancer do Estado de Sao Paulo ICESP, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, Brazil, 10. Comprehensive Center for Precision Oncology, Universidade de Sao Paulo, São Paulo, Brazil.

e-mail:
fernandachiarot@usp.br
lara.termini@hc.fm.usp.br

STUDY DESIGN

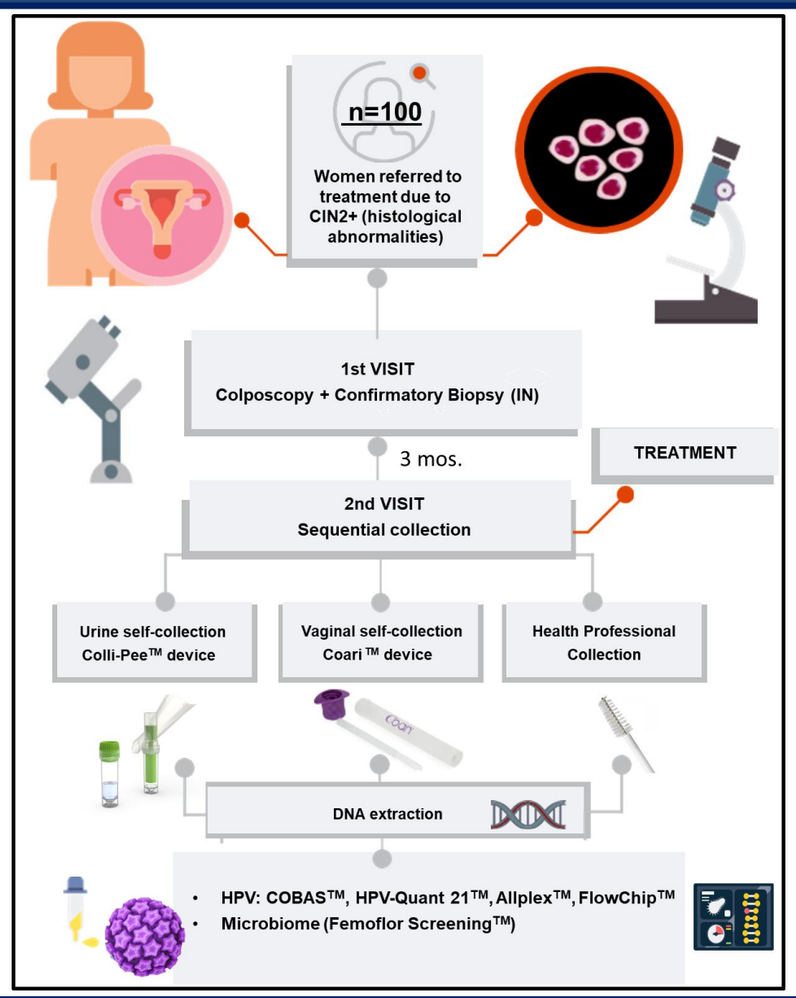

Figure 1. Video QR-CODE Patient's Explanatory video

INTRODUCTION

High-risk HPV (hrHPV) DNA detection is an effective strategy to prevent cervical cancer. Self-collection methods could help reduce incidence, especially among women with limited healthcare access.

Benefits of Self-Collection:

Urine and vaginal self-collection methods are less invasive and widely accepted. They offer viable alternatives for women who avoid conventional screenings due to factors like fear, cultural beliefs, physical limitations, or lack of access to healthcare facilities.

Study Objective:

This study assesses the association and concordance of CIN2+ lesions with hrHPV and microbiome across different sample types: urine **(U)**, vaginal self-collection **(SC)**, and healthcare professional collection **(PC)**.

MATERIALS & METHODS

Cross-sectional study with 100 women aged >21 referred for colposcopy due to CIN2+ histological findings

Self-Collection Guidance - Patients were shown an instructional video to guide them through the self-collection steps (**Fig. 1 – QR CODE**)

Sample Collection - Three sample types were collected sequentially in the same visit:

Urine (U) ➡ Vaginal Self-Collection (SC) ➡ Healthcare Professional Collection (PC)

HPV Testing - COBAS™ 4800 (Roche), HPV-Quant 21™ (DNA-Technology LLC), Allplex™ (Seegene), Flow Chip™ (Mobius Life Science). **Microbiome Analysis -** Femoflor Screening™ platform (DNA-Technology LLC).

Statistical Analysis - Descriptive statistics: Absolute and relative frequencies, 95% CI, and mean ± SD. Concordance tests: Cohen's Kappa and McNemar's test for agreement assessment.

RESULTS

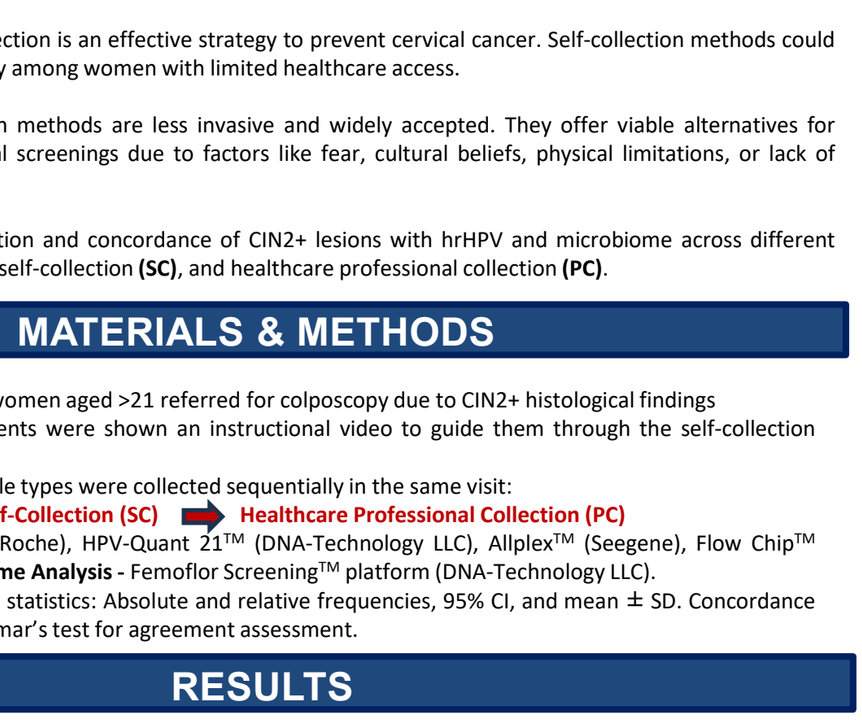

Figure 2 conclusion:

- All four methods showed consistent detection of HPV16, HPV18, and OHR types. HPV16 was detected in ~35–40%, OHR in 60–70%, and HPV18 in <10% of samples. Urine, self-collected, and clinician-collected samples yielded similar results, indicating flexibility in sample choice.

Figure 2. HPV positivity rate (%) by detection method and sample collection type. OHR: other High Risk HPV group: 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. Vertical lines at the center of the bars represent the 95% confidence interval

RESULTS

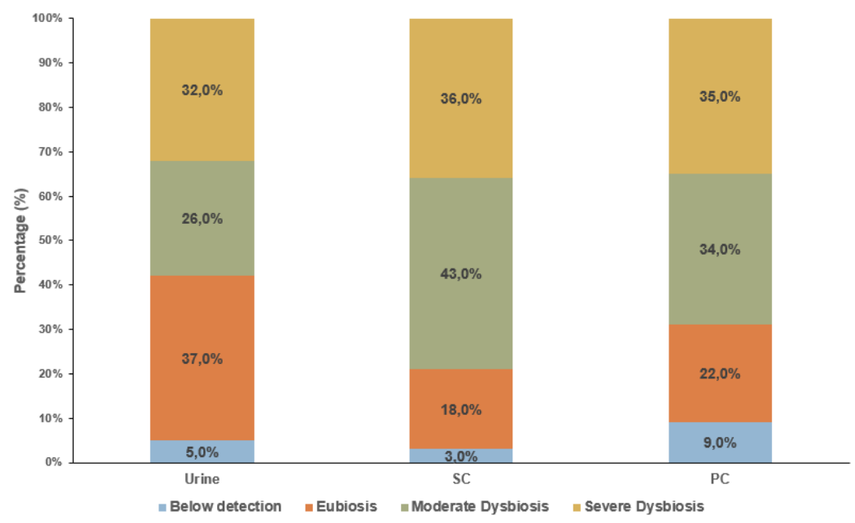

Figure 3 conclusion:

- Urine samples showed the highest rate of eubiosis, while dysbiosis (moderate and severe) was more frequent in self-collected samples, suggesting potential microbiota differences according to sample type

Figure 3. Distribution of normobiosis and dysbiosis by sample collection method. Vertical lines at the center of the bars represent the 95% confidence interval

Table 1. Concordance between **urine**, vaginal self-collection **(SC)**, and healthcare professional collection **(PC)** samples for microbiome components and sexually transmitted infections (STIs)

	Urine *vs* SC		Urine *vs* PC		SC *vs* PC	
Microbiome Components and STIs	**kappa**	**p-value**	**kappa**	**p-value**	**kappa**	**p-value**
Lactobacillus spp.	0,878	<0.001	0,841	<0.001	0,735	<0.001
Lactobacillus crispatus	0,777	<0.001	0,720	<0.001	0,771	<0.001
Lactobacillus gasseri	0,757	<0.001	0,718	<0.001	0,861	<0.001
Lactobacillus iners	0,908	<0.001	0,888	<0.001	0,886	<0.001
Lactobacillus jensenii	0,721	<0.001	0,735	<0.001	0,928	<0.001
Streptococcus agalactiae	0,693	<0.001	0,693	<0.001	0,864	<0.001
Chlamydia trachomatis	1,000	<0.001	0,884	<0.001	0,884	<0.001
Mycoplasma genitalium	0,481	<0.001	0,481	<0.001	1,000	<0.001
Herpes simplex virus type 1	1,000	<0.001	1,000	<0.001	1,000	<0.001
Herpes simplex virus type 2	0,823	<0.001	0,427	<0.001	0,559	<0.001
Citomegalovirus	0,135	0,176	0,481	<0.001	0,421	<0.001
Candida spp.	0,885	<0.001	0,853	<0.001	0,743	<0.001
Candida albicans	0,795	<0.001	0,662	<0.001	0,492	<0.001
Candida glabrata	1,000	<0.001	1,000	<0.001	1,000	<0.001
Gardnerella vaginalis + Prevotella bivia + Porphyromonas spp.	0,643	<0.001	0,351	<0.001	0,682	<0.001
Mycoplasma hominis	0,952	<0.001	0,953	<0.001	0,952	<0.001
Ureaplasma spp.	1,000	<0.001	0,937	<0.001	0,937	<0.001

Table 1. conclusion: Overall, high levels of agreement were observed, except for *Cytomegalovirus* when comparing urine with SC samples, and for *Gardnerella vaginalis + Prevotella bivia + Porphyromonas spp.* when comparing urine with PC samples.

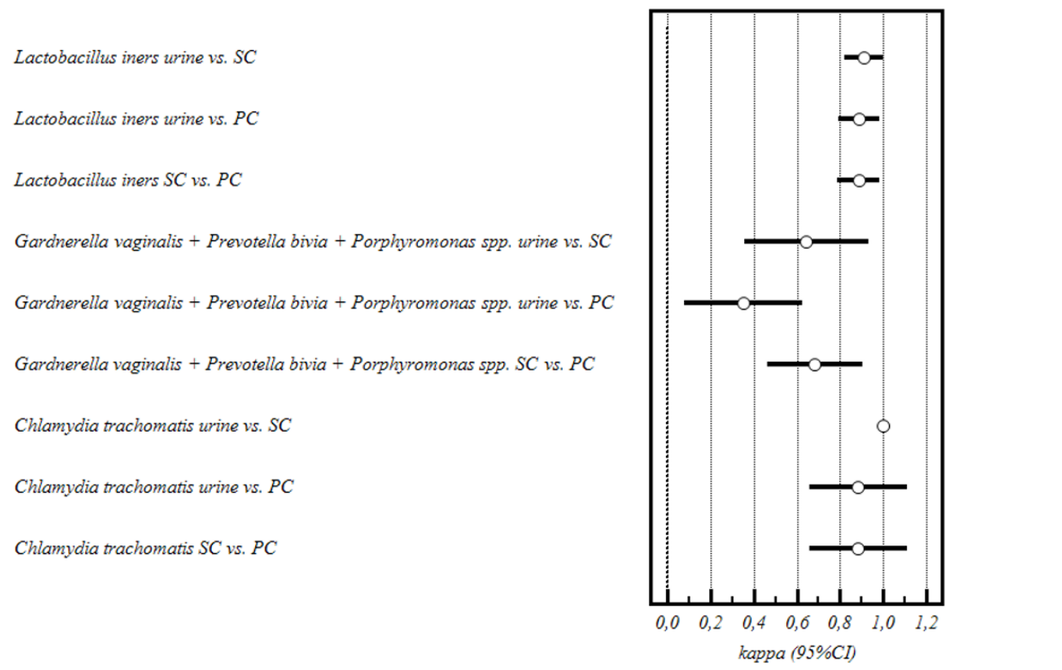

Figure 4. Agreement (Kappa, 95% CI – *Confidence Interval*) between **urine**, vaginal self-collection **(SC)**, and professional collection samples **(PC)** for detection of microbiome components and sexually transmitted infections (STIs).

Figure 4 conclusion: Cohen's kappa agreement (κ; 95% CI) was assessed between urine, SC, and PC samples. Substantial to almost perfect agreement was observed for *Lactobacillus iners* and *Chlamydia trachomatis*, while moderate agreement was observed for bacterial vaginosis–associated species (*Gardnerella vaginalis, Prevotella bivia,* and *Porphyromonas spp.).*

SUPPORT AND ACKNOWLEDGMENTS

Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP 2022/16783-3); Laboratórios Fleury (Brazil) (NP-679), Instituto de Pesquisa em Oncologia Ginecológica – IPOG (Brazil); Grupo Kolplast (Brazil), DNA-Technology LLC, Biomedica Eq. Médicos, Qiagen, Seegene, Mobius Life Science.

REFERENCES

(1) Arbyn et al., 2014; **(2)** Polman et al., 2019; **(3)** Lorenzi et al., 2019; **(4)** Lorenzi et al., 2022; **(5)** Leeman et al., 2017; **(6)** Pattyn et al., 2019; **(7)** Van Keer et al., 2018; **(8)** Mitra et al., 2015; **(9)** Mitra et al., 2016; **(10)** Castanheira et al., 2021; **(11)** Castanheira et al., 2025.