COMPARATIVE ANALYSIS OF HPV GENOTYPING AND MICROBIOME PROFILE IN UTERINE CERVIX SAMPLES OBTAINED BY URINE, SELF-COLLECTION AND HEALTHCARE PROFESSIONALS
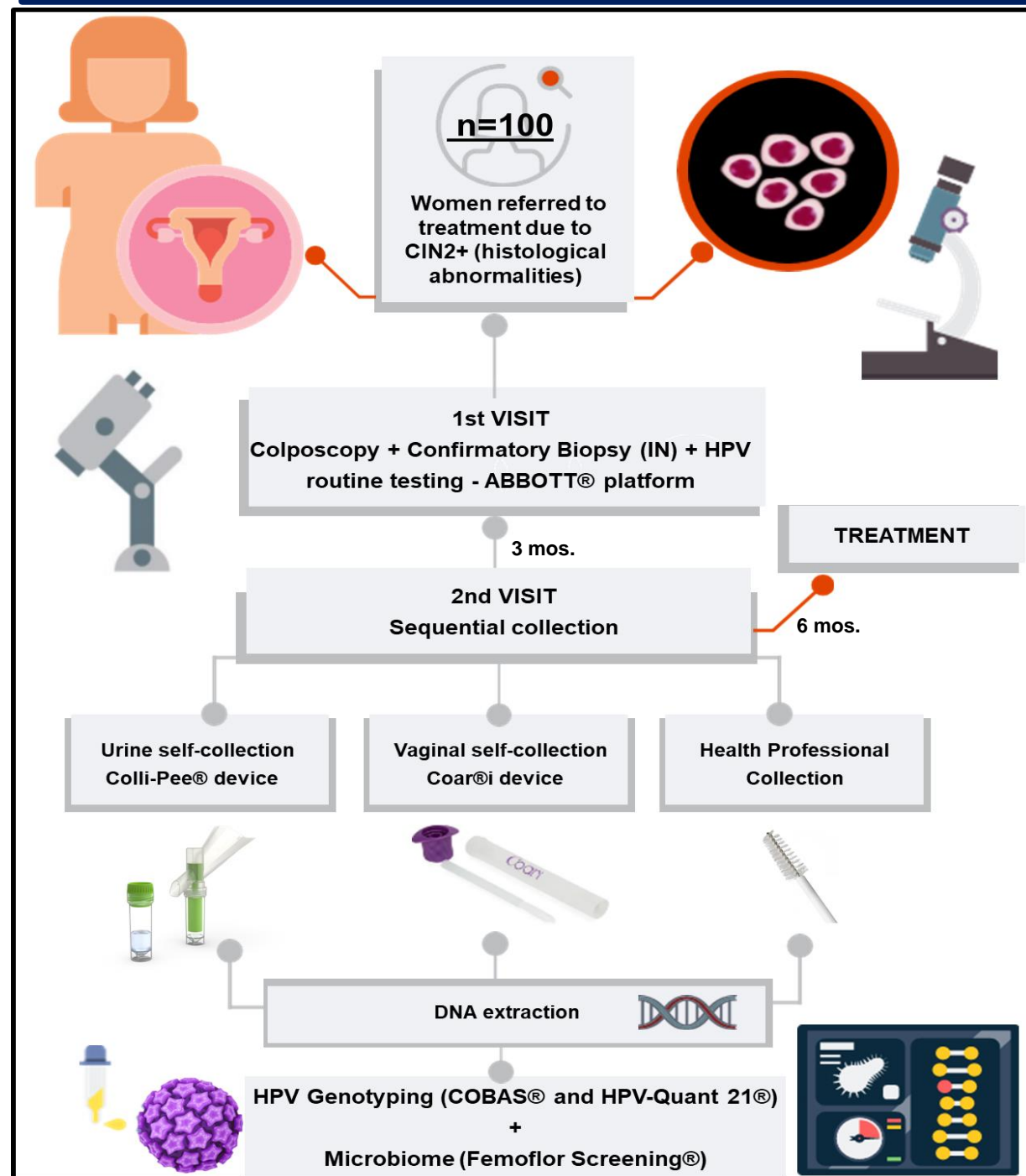
Cristina Paula Castanheira[1,2], Noely Paula Cristina Lorenzi[3], Fernanda Dahrouge Chiarot[4], Maricy Tacla[1], Adhemar Longatto-Filho[5], Edmund C Baracat[6], Luisa Lina Villa[7,8], Gustavo A. R. Maciel[6], **Lara Termini***[7,8]

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

e-mail: terminilara@gmail.com
lara.termini@hc.fm.usp.br

ABSTRACT

n=100

Women referred to treatment due to CIN2+ (histological abnormalities)

1st VISIT
Colposcopy + Confirmatory Biopsy (IN) + HPV routine testing - ABBOTT® platform

3 mos. → TREATMENT

2nd VISIT
Sequential collection
6 mos.

Urine self-collection Colli-Pee® device | Vaginal self-collection Coar®i device | Health Professional Collection

DNA extraction

HPV Genotyping (COBAS® and HPV-Quant 21®) + Microbiome (Femoflor Screening®)

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 STI positivity across different sample types—urine (U), vaginal self-collection (VSC), and healthcare professional collection (HPC). Vaginal microbiome analysis was also conducted.

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 (VSC)** ⟹ **Healthcare Professional Collection (HPC)**

Testing - HPV DNA Testing (**Fig. 2**) performed using COBAS® 4800 (Roche) and HPV-Quant 21® (DNA-Technology LLC)
Microbiome Analysis conducted with the 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
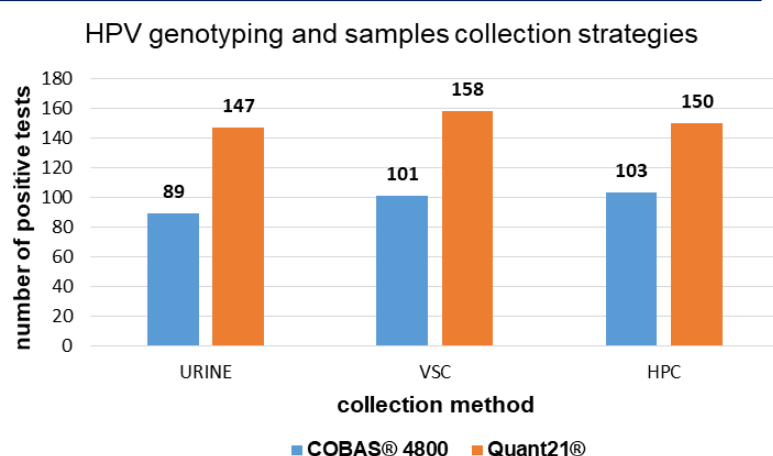

Figure 1. Video QR-CODE
Patient's Explanatory video

HPV genotyping and samples collection strategies

Figure 2. Overall HPV positive tests (n)

RESULTS

Table 1. Acceptance of sample collection techniques and explanatory video among participants of the study.

Choice of collection method, n;(CI95)		
Vaginal Self-Collection	17	17,0 (10,6-25,2)
Healthcare Professional Collection	14	14,0 (8,3-21,8)
Urine Collection	53	53,0 (43,2-62,6)
Indifferent	16	16,0 (9,8-24,1)
Opinion on the educational video, n;(CI95)		
Video Greatly Aided Understanding	100	-
Health services should have more educational videos, n;(CI95)		
Yes	100	-
No	0	-
Reasons for approving or disapproving the educational video, n;(CI95)		
Would Like to Watch Video with Professional Assistance	38	38,0 (28,9-47,7)
Video Increased Comfort Using Collector and Brush	62	62,0 (52,3-71,1)

Table 1 conclusion. Urine collection was the most accepted technique (53%), followed by vaginal self-sampling collection (17%). The use of the educational video was approved by 100% of the participants, underscoring the importance of the use of this visual tool in health services.

Table 2. COBAS® HPV test agreement analysis between different sample collection strategies.

	C+	D+	C-	D-	Kappa	p-value†	p-value‡
HPV16							
VSC vs HPC	38	2	60	1	0.960	<0.001	0.500
URINE vs HPC	37	3	59	2	0.950	<0.001	0.600
URINE vs VSC	39	1	58	2	0.970	<0.001	0.450
HPV18							
VSC vs HPC	3	1	93	2	0.850	0.002	0.980
URINE vs HPC	2	1	94	1	0.830	0.003	0.970
URINE vs VSC	3	0	95	1	0.880	<0.001	0.880
HPVOHR							
URINE vs HPC	49	5	40	2	0.900	<0.001	0.660
URINE vs VSC	51	4	39	3	0.910	<0.001	0.550
VSC vs HPC	56	3	38	1	0.890	<0.001	0.720

† Kappa p-value; ‡ McNemar test p-valor; C+: Concordant (Positive); D+: Discordant (Positive); C-: Concordant (Negative); D-: Discordant (Negative); VSC: Vaginal Self-collection; HPC: Health Professional collection. HPVOHR: Other High-risk HPV.

Table 2 conclusion. We observed high level of agreement of HPV types detected across all sample collection techniques.

Table 3. COBAS® *vs* Quant21® test agreement analysis between different sample collection techniques for HPV16 and 18 types.

	C+	D+	C-	D-	Kappa	p-value†	p-value‡
HPV16							
URINE	37	0	59	4	0.916	<0.001	0.125
VSC	40	0	59	1	0.979	<0.001	0.999
HPC	39	0	60	1	0.979	<0.001	0.999
HPV18							
URINE	2	1	97	0	0.795	<0.001	0.999
VSC	4	0	92	4	0.648	<0.001	0.125
HPC	3	0	93	4	0.582	<0.001	0.125

† Kappa p-value; ‡ McNemar test p-valor; C+: Concordant (Positive); D+: Discordant (Positive); C-: Concordant (Negative); D-: Discordant (Negative); VSC: Vaginal Self-collection; HPC: Health Professional collection.

Table 3 conclusion. We observed high level of agreement of HPV16 and 18 genotyping (COBAS® vs Quant21®) and sample collection techniques tested.

Table 4. Microbiome and Sexually Transmitted Infections (STI) analysis including concordant and discordant cases.

	C+	D+	C-	D-	Kappa	p-value†	p-value‡
Streptococcus Agalactiae							
URINE vs VSC	5	1	91	3	0.693	0.001	0.625
URINE vs HPC	5	1	91	3	0.693	0.001	0.625
VSC vs HPC	7	1	91	1	0.864	0.001	0.999
Chlamydia trachomatis							
URINE vs VSC	5	0	95	0	1.000	<0.001	1.000
URINE vs HPC	4	1	95	0	0.884	0.001	1.000
VSC vs HPC	4	1	95	0	0.884	0.001	1.000
Mycoplasma genitalium							
URINE vs VSC	2	3	94	1	0.481	0.001	0.625
URINE vs HPC	2	3	94	1	0.481	0.001	0.625
VSC vs HPC	3	0	97	0	1.000	<0.001	0.999
Herpes simplex 1 virus							
URINE vs VSC	1	0	99	0	1.000	<0.001	0.999
URINE vs HPC	1	0	99	0	1.000	<0.001	0.999
VSC vs HPC	1	0	99	0	1.000	<0.001	0.999
Herpes simplex 2 virus							
URINE vs VSC	5	2	93	0	0.823	0.001	0.500
URINE vs HPC	2	5	93	0	0.427	0.001	0.062
VSC vs HPC	2	3	95	0	0.559	0.001	0.250
Cytomegalovirus							
URINE vs VSC	1	3	90	6	0.138	0.160	0.508
URINE vs HPC	1	3	94	2	0.26	0.005	0.999
VSC vs HPC	3	4	93	0	0.582	0.001	0.125
Candida spp							
URINE vs VSC	8	22	55	15	0.056	0.573	0.324
URINE vs HPC	5	25	67	3	0.157	0.108	<0.001
VSC vs HPC	4	19	73	4	0.158	0.105	0.003
Mycoplasma hominis							
URINE vs VSC	29	2	69	0	0.952	0.001	0.500
URINE vs HPC	30	1	68	1	0.953	0.001	0.999
VSC vs HPC	29	0	69	2	0.952	0.001	0.500
Ureaplasma (urealyticum + parvum)							
URINE vs VSC	61	0	37	2	0.958	0.001	0.500
URINE vs HPC	57	4	38	1	0.896	0.001	0.375
VSC vs HPC	58	5	37	0	0.896	0.001	0.062
Flora Conclusion *							
URINE vs VSC	58	4	19	19	0.472	0.001	0.003
URINE vs HPC	59	3	16	22	0.413	0.001	<0.001
VSC vs HPC	71	6	13	10	0.519	0.001	0.454

* normal flora *vs* dysbiosis

Table 4 conclusion. Considering the different collection strategies, results for *Mycoplasma hominis* and *Ureaplasma* showed strong concordance, while flora data (dysbiosis *vs* normal flora) exhibited reasonable concordance. On the other hand, *Cytomegalovirus* and *Candida* spp. showed weak and low concordance, respectively.

MAIN CONCLUSIONS

Urine collection and vaginal self-sampling were the most accepted techniques.

The detection of most STI, including HPV, and microbiome components, showed high concordance among the collecting strategies.

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 al., 2016; (10) Castanheira et al., 2021