

Feasibility and Performance of Multiple Urinary HPV Detection Assays in Women with Known Cervical HPV Status: A Pilot Study

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Abstract

Background:

Human papillomavirus (HPV) DNA testing is effective for cervical cancer screening, yet conventional cervical sampling poses difficulties in certain populations. Urine-based HPV testing offers a non-invasive option, yet its accuracy depends on sample collection and on assay sensitivity. This pilot study explored urinary HPV DNA across different collection methods and assays.

Methods:

In this pilot cross-sectional study, 253 women attending outpatient clinics were screened for cervical HPV DNA; 15 (5.9%) tested positive and were recruited alongside 15 age-matched HPV-negative controls. First-void urine was collected using disposable urine culture bottles and the Colli-Pee device. HPV DNA detection was assessed using Hybrid Capture-2 (HC-2), polymerase chain reaction (PCR), and line probe assay (LPA). Colposcopy was performed in all HPV-positive women to evaluate associated cervical abnormalities.

Results:

No urinary HPV DNA was detected in samples obtained using disposable urine culture bottles (0/30). The Colli-Pee device showed higher detection rates among HPV-positive women ($n = 15$); urinary HPV was detected in 9/15 (60%) by PCR, 7/15 (46.7%) by LPA, and 3/15 (20%) by HC-2. None of the HPV-negative women showed detectable HPV DNA in urine samples across all assays and collection methods. Colposcopy revealed normal findings in 13 (86.7%), LSIL in 1 (6.7%), and HSIL in 1 (6.7%).

Conclusion:

Urinary HPV DNA detection is influenced by the sampling methods and assay sensitivity. Standardized first-void urine collection using Colli-Pee, combined with PCR, enhances detection. While cervical sampling remains the reference standard, optimized urine-based testing offers a promising, non-invasive complementary approach for HPV screening that requires validation in larger prospective studies and needs further assessment.

Introduction

Cervical cancer continues to represent a major global public health concern, particularly in low- and middle-income countries, where it substantially contributes to cancer-related illness and death [1]. Persistent infection with high-risk human papillomavirus (HPV) is the main causative factor and is responsible for over 95% of cases [2].

Urine-based HPV DNA testing has emerged as a non-invasive alternative to cervical sampling. First-void urine contains exfoliated cervical and vaginal cells, making it a suitable specimen for HPV detection [3,

4]. This method has the potential to enhance acceptability and participation in screening initiatives. However, heterogeneity in urine collection techniques, the absence of standardized protocols, and a generally lower DNA yield compared with cervical samples have constrained its integration into routine clinical practice [5].

The development of standardized urine collection devices, such as the Colli-Pee, seeks to address these challenges by selectively capturing the first-void urine fraction and stabilizing the nucleic acids [3, 4]. Although initial findings are encouraging, data comparing urinary HPV detection in both HPV-positive and HPV-negative women using standardized collection methods remain restricted. Limited Indian studies are available comparing different urinary HPV detection assays using standardized first-void urine collection [6].

Consequently, this pilot study set out to investigate urinary HPV DNA detection among HPV-positive women and to assess exploratory evaluation in both age-matched HPV-positive and HPV-negative cohorts using various urine collection approaches and molecular assays, including Hybrid Capture-2, Polymerase Chain Reaction, and Line Probe Assay.

Materials and Methods

This pilot exploratory cross-sectional study was conducted in the Department of Obstetrics and Gynaecology, Microbiology and Laboratory Oncology, All India Institute of Medical Sciences (AIIMS), New Delhi, from 19 January 2024 to 20 November 2025. The study design and workflow are shown in (Fig. 1).

Figure 1- Study Schema

OPD- Outpatient Department

Study Population

Women aged > 25 years, attending the outpatient department, were recruited for the study. Exclusion criteria were prior cervical cancer screening within three years (cytology) or vaginal bleeding. All the participants were informed that the confidentiality of the data would be maintained. Ethical approval for the study was obtained from the Institutional Ethical Committee (IEC) with reference number: AIIMSA00216/13.12.2023, RT-07/18.01.2024. The study was conducted in accordance with ICH-GCP (International Council for Harmonisation - Good Clinical Practice), ICMR (Indian Council of Medical Research), and New Drugs and Clinical Guidelines-2019. Written informed consent was obtained from all study participants before enrolment.

Sample Collection

A sample size of 253 women attending outpatient services was screened for cervical HPV DNA detection using the Digene HC2 Assay following the manufacturer's protocol (Qiagen, 2019), out of

which 15 tested positive. From the screened population, these 15 HPV-positive women and 15 age-matched HPV-negative women were selected for exploratory urine-based HPV assessment. Urine sampling was performed during the same study visit immediately after participant enrolment and confirmation of eligibility.

Urine Sample Collection

First-void urine samples were collected from both groups (n = 30) in both disposable urine culture bottle (approx. 20-30ml) on one morning, which was followed by the Colli-Pee collection device (20ml collection volume) on the subsequent morning [Figure 2 (a) and (b)]. Therefore, both collections represented first-void urine samples, and patients were informed about the urine collection methods.

(a) Disposable urine culture bottle is used for routine sample collection. **(b)** The Colli-Pee® device is designed for standardised first-void urine collection.

Sample Processing

For comparative evaluation, aliquots from each urine sample were processed independently for Hybrid Capture-2 (HC-2), PCR and Line Probe Assay (LPA). DNA extraction was therefore done separately for each assay using the protocol and kit recommended for that respective assay.

The samples were analysed using: -

1. Hybrid Capture-2 - DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Germany; Cat. No. 51304). Briefly, 200 µL of urine was processed according to the manufacturer's instructions and eluted in 50 µL Buffer AE before HC-2 analysis. HPV DNA detection was performed using the Digene Hybrid Capture-2 assay (Qiagen, USA) as per the manufacturer's instructions. The assay detects 13 high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) using RNA-DNA hybridization with chemiluminescent signal amplification.
2. Polymerase Chain Reaction - DNA was extracted independently using the TRUPCR Tissue DNA Extraction Kit (3B BlackBio Biotech India Ltd., India) according to the manufacturer's protocol. Briefly, urine sediment underwent Proteinase K digestion followed by silica-column purification. Purified DNA was eluted in 35–50 µL elution buffer and quantified using NanoDrop spectrophotometry. DNA concentrations ranged between 7–20 ng/µL, with an A260/A280 ratio of 1.9–2.0. HPV detection was performed using the TRUPCR® HPV High-Risk Genotyping Assay on the Bio-Rad CFX96 Real-Time PCR System. The assay detects 14 high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) by targeting the E6/E7 oncogenes using TaqMan hydrolysis probes. Each reaction consisted of 25 µL master mix and 5 µL DNA, giving a total reaction volume of 30 µL. Thermal cycling consisted of 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds. Positive and negative controls were included in every run. According to the manufacturer's specifications, the assay has an analytical sensitivity (limit of detection) of approximately 100 IU/mL.

3. Line probe assay – This was performed using the INNO-LiPA HPV Genotyping Extra II assay (Fujirebio Europe, Belgium) according to the manufacturer's instructions. DNA was extracted from first-void urine collected via the Colli-Pee using the QIAamp DNA Mini Kit (Qiagen, Germany) as per the manufacturer's instructions and was amplified using SPF10 primers targeting a conserved 65-bp fragment of the HPV L1 region. Amplified products were hybridized to genotype-specific oligonucleotide probes immobilized on nitrocellulose strips using reverse hybridization. The assay detects 32 HPV genotypes, including 13 high-risk, 7 probable high-risk and 12 low-risk HPV types. Hybridization results were interpreted using LiRAS software.

Outcome Measures

The primary outcome was an exploratory assessment of urinary HPV DNA detection compared with cervical HPV DNA testing as standard, across different collection methods and molecular assays.

Secondary outcomes included the feasibility assessment of urine-based HPV testing as compared to cervical HPV DNA testing in both HPV-positive and HPV-negative women. Following sample collection, participants were directly asked to rate ease of urine and cervical sampling with four response categories (easy, moderately easy, moderately difficult, difficult).

All 15 participants who were HPV-positive on cervical sampling were referred for colposcopy examination. Directed biopsies were obtained from abnormal areas as per the Swede score assessment system, and subsequent management was provided based on colposcopy and histopathological findings.

Statistical Analysis

The sample size for the screening phase was calculated using the formula $n = 4pq/d^2$. Based on the HPV prevalence of 8% reported by Sudhakaran et al. [6] and a 5% absolute precision at 95% confidence level, yielding the minimum required sample size of 113. Exact binomial 95% confidence intervals (Clopper-Pearson) were calculated. To enhance study feasibility and to overcome potential attrition, 253 participants were included. The exploratory assessment was conducted on a subset of participants, which included 15 HPV-positive women and 15 age-matched HPV-negative controls. This component of the study was exploratory in nature and may be considered a pilot evaluation of urinary HPV DNA testing.

Data were analyzed using STATA version 15.0. Categorical variables were expressed as frequencies and percentages. Exploratory detection characteristics, including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), were calculated descriptively using cervical HPV DNA testing as the gold standard. Fisher's exact test was used to compare baseline characteristics between HPV-positive and HPV-negative groups. Inter-assay comparisons of detection rates were not performed.

due to the exploratory nature of this pilot study and small size. A p-value < 0.05 was considered statistically significant.

Results

Among the 253 women screened, 15 (5.9%) were found positive for cervical HPV DNA. Further analysis included a total of 30 women, out of whom 15 were HPV-positive, and 15 were age-matched HPV-negative women, who served as controls for diagnostic performance evaluation.

Baseline Characteristics of Study Participants

The mean age was comparable in both groups (31.9 ± 6.4 years vs 31.7 ± 5.9 years; $p = 0.930$). There were no significant differences between groups regarding education level, age at menarche, contraceptive use, comorbidities, or HIV status. All participants were non-smokers, and none had previously undergone cervical cancer screening (Table 1).

Table 1
Baseline characteristics of study participants (n = 30)

Variable	HPV-Positive (n = 15)	HPV-Negative (n = 15)	p-value
Age (in years)			
25–29	5 (33.3%)	5 (33.3%)	0.930
30–34	6 (40.0%)	6 (40.0%)	
35–39	2 (13.3%)	2 (13.3%)	
≥ 40	2 (13.3%)	2 (13.3%)	
Mean ± SD: 31.9 ± 6.4 vs 31.7 ± 5.9			
Education level			
No Formal Schooling	0 (0.0%)	3 (20.0%)	0.062
Primary or below	2 (13.3%)	4 (26.7%)	
Secondary	7 (46.7%)	2 (13.3%)	
Graduate	5 (33.3%)	5 (33.3%)	
Postgraduate & above	1 (6.7%)	1 (6.7%)	
Occupation			
Homemaker	14 (93.3%)	12 (80.0%)	0.598
Unskilled	1 (6.7%)	2 (13.3%)	
Skilled	0 (0.0%)	1 (6.7%)	
Parity			
Nulliparous	9 (60.0%)	7 (46.7%)	0.687
Para 1	3 (20.0%)	5 (33.3%)	
Para 2	1 (6.7%)	3 (20.0%)	
Para ≥ 3	2 (13.3%)	0 (0.0%)	
Smoking history	15 (100%)	15 (100%)	N/A
OCP = oral contraceptive pill; IUCD = intrauterine contraceptive device; N/A = not applicable (no variation).			
p-values calculated using Fisher’s exact test for categorical variables and independent samples t-test for continuous variables.			
Age-matched controls (HPV-negative, n = 15) selected from the screened cohort of 234 HPV-negative women, matched 1:1			

Variable	HPV-Positive (n = 15)	HPV-Negative (n = 15)	p-value
Age (in years)			
Non-smoker			
Contraceptive use			
None	14 (93.3%)	10 (66.7%)	0.169
OCP	0 (0.0%)	2 (13.3%)	
IUCD	1 (6.7%)	1 (6.7%)	
Implant/Other	0 (0.0%)	2 (13.3%)	
History of cervical cancer screening	15 (100%)	15 (100%)	N/A
Never screened			
Medical comorbidities			
Absent	6 (40.0%)	8 (53.3%)	0.715
Present	9 (60.0%)	7 (46.7%)	
HIV status			
Negative	14 (93.3%)	15 (100%)	1.000
Positive	1 (6.7%)	0 (0.0%)	
OCP = oral contraceptive pill; IUCD = intrauterine contraceptive device; N/A = not applicable (no variation).			
p-values calculated using Fisher’s exact test for categorical variables and independent samples t-test for continuous variables.			
Age-matched controls (HPV-negative, n = 15) selected from the screened cohort of 234 HPV-negative women, matched 1:1			

Urinary HPV DNA Detection Using Disposable Urine Culture Bottle-based Collection

First-void urine samples collected using the disposable urine culture bottle showed no HPV DNA positivity in the HPV-positive group (n = 15) with cervical HPV infection (0/15, 0%), using the Hybrid Capture-2 (HC-2), Polymerase Chain Reaction (PCR) and Line Probe Assay (LPA).

Urinary HPV DNA Detection Using Colli-Pee Device

In contrast, first-void urine samples collected using the Colli-Pee device among the 15 women with positive cervical HPV infection showed (Table 2):

Table 2

Exploratory detection characteristics of urinary HPV DNA assays for HPV and CIN (LSIL/HSIL) detection

	Method	Sensitivity (%)	95% CI for Sensitivity	Specificity (%)	PPV (%)	NPV (%)
HPV Detection (Reference: Cervical HPV) (n = 30)	PCR	60.0 (9/15)	32.3–83.7%	100 (15/15)	100	71.4
	LPA	46.7 (7/15)	21.3–73.4%	100 (15/15)	100	65.2
	HC-2	20.0 (3/15)	4.3–48.1%	100 (15/15)	100	55.6
CIN Detection (LSIL/HSIL) (Reference: Histopathology) (n = 15)	PCR	100.0 (2/2)	15.8–100%*	46.2 (6/13)	22.2 (2/9)	100.0 (6/6)
	LPA	100.0 (2/2)	15.8–100%*	61.5 (8/13)	28.6 (2/7)	100.0 (8/8)
	HC-2	100.0 (2/2)	15.8–100%*	92.3 (12/13)	66.7 (2/3)	100.0 (12/12)
Diagnostic performance of urinary HPV DNA detection assays compared with cervical HPV testing and histopathology. For HPV detection, cervical HPV DNA testing was used as the reference standard (n = 30; HPV-positive = 15, HPV-negative = 15). For CIN detection, histopathology-confirmed LSIL/HSIL lesions were used as the reference standard (n = 15 HPV-positive women). Disposable bottle collection achieved 0% sensitivity for both HPV and CIN detection across all assays. None of the 15 HPV-negative women demonstrated detectable HPV DNA in urine samples collected using the urine culture container or Colli-Pee methods across all three assays, indicating 100% specificity in this pilot cohort (95% CI: 78.2–100%). Ninety-five % CIs calculated using the exact Clopper-Pearson method. Wide confidence intervals reflect the exploratory, pilot nature of the study. NPV values reflect the 1:1 HPV-positive to HPV-negative ratio in this pilot cohort and are not generalisable to screening populations.						
*Wide CI reflects n = 2 CIN-positive cases; results should be interpreted with caution.						
PPV = positive predictive value; NPV = negative predictive value; LSIL = Low grade squamous intraepithelial lesion; HSIL = High grade squamous intraepithelial lesion; PCR = Polymerase Chain Reaction; LPA = Line Probe Assay; HC-2 = Hybrid Capture-2; CI = Confidence interval						

- PCR detected HPV DNA in 9/15 (60%) cases
- LPA detected HPV DNA in 7/15 (46.7%) cases
- HC-2 detected HPV DNA in 3/15 (20%) cases

A visual summary of detection rates across both collection methods is provided (Supplementary material, Figure S1 – Graphical abstract). This represents a substantial improvement in detection across assays compared to disposable urine culture bottle-based collection, where no cases were detected.

While the Colli-Pee device increased detection rates, urinary HPV DNA was still not found in some individuals who were cervical HPV-positive, indicating that urine-based testing does not provide complete sensitivity.

Urinary HPV Detection in HPV-Negative Women

None of the 15 HPV-negative women demonstrated detectable HPV DNA in urine samples collected using the urine culture container or Colli-Pee methods across all three assays (PCR, HC-2, and LPA), indicating 100% specificity in this pilot cohort.

Exploratory Assessment of Urinary HPV Detection

Using cervical HPV positivity as the reference standard (n = 30), exploratory assessment metrics were calculated based on these detection rates, where PCR demonstrated the higher detection rate (60%), followed by LPA (46.7%) and HC-2 (20%), while all assays showed 100% specificity. Positive predictive value (PPV) was 100% for all Colli-Pee assays, while negative predictive value (NPV) varied according to assay sensitivity, being highest for PCR (71.4%) and lowest for HC-2 (55.6%) (Table 2).

Using histopathology as the reference standard for CIN detection (n = 15 HPV-positive women; CIN-positive = 2, CIN-negative = 13), all three Colli-Pee assays achieved 100% sensitivity and 100% NPV, indicating that no CIN case was missed. Specificity for CIN detection was highest with HC-2 (92.3%) followed by LPA (61.5%) and PCR (46.2%). Disposable bottle collection achieved 0% sensitivity for both HPV and CIN detection across all assays (Table 2).

Colposcopy and Histopathological Findings

The integrated colposcopy assessment shows that most patients demonstrated low-grade colposcopy features. Based on the composite Swede score, the majority (12 patients, 80%) fell within the 0–4 low-risk category, two patients were within the 5–7 score category, and only one patient (6.7%) reached the 8–10 high-risk threshold, indicating a low overall prevalence of significant lesions. On the HPE report, the majority were reported as normal (13/15), only 1 case out of 15 was reported as low-grade squamous intraepithelial lesion (LSIL) and 1 case was reported as high-grade squamous intraepithelial lesion (HSIL). These findings suggest an overall predominance of low-grade pathology in the study population. The distribution demonstrates strong concordance between clinical impression and histopathology. Both LSIL and HSIL cases were consistently detected using Colli-Pee urine collection across all three assays (100%), whereas disposable culture bottle-based urine samples failed to detect these cases (0%).

HPV Genotype Detection

HPV genotyping was performed on Colli-Pee collected first-void urine samples of the 15 HPV-positive women using PCR and LPA, and their detection profiles were compared.

PCR predominantly detected high-risk HPV genotypes including HPV16, HPV18, HPV31 and HPV35. In contrast, LPA identified a wider spectrum of HPV genotypes, including additional high-risk types such as HPV33 and HPV45 and low-risk types such as HPV6, HPV44, HPV54, HPV61, not detected by PCR. Many

HPV-positive women were found to possess more than one HPV genotype, resulting in the total number of genotype detections exceeding the total number of HPV-positive patients. The distribution of HPV genotypes detected by PCR and LPA is shown in (Fig. 3).

PCR = Polymerase Chain Reaction; LPA = Line Probe Assay

Feasibility of Sampling Methods

Urine-based sampling showed greater ease of collection compared to cervical sampling. A larger share of participants rated urine collection as easy and moderately easy (100.0%) when compared with the ease of cervical sampling (80.0%), making it more feasible for the patients (Fig. 4). Together, these results suggest that urine-based sampling is more feasible for women compared to cervical sampling, potentially reflecting a preference for non-invasive methods.

Discussion

This pilot study explored urinary HPV DNA detection in women with known HPV positivity and compared it with clinician-obtained cervical samples, focusing particularly on how urine collection methods and assay type influence test performance. The results show that HPV detection in urine may be influenced by both the sampling technique and the analytical characteristics of the assay employed. No HPV-negative participant had detectable HPV DNA in any urine sample, regardless of assay or collection method.

An important finding in this study was the complete absence of detectable HPV DNA in urine specimens collected in disposable urine culture containers, even though cervical HPV infection had been confirmed. This observation aligns with previous research indicating low sensitivity of urine-based HPV testing when first-void urine is inadequately captured or when no preservative is added [7, 8]. Plausible reasons include dilution of exfoliated cervical cells, lack of a stabilizing medium, and degradation of viral DNA during transport and storage.

In contrast, implementing the Colli-Pee device led to higher HPV detection rates. This device is engineered to collect the first-void portion of urine, which is known to harbour the greatest concentration of exfoliated cervical cells, and it contains a preservation medium that stabilizes nucleic acids. This is consistent with studies demonstrating that first-void urine collection devices enhance the recovery of HPV DNA by capturing the initial urinary stream and stabilizing nucleic acids [9, 10]. These findings suggest standardized first void-urine collection, even with enhanced detection using Colli-Pee, still misses a subset of cervical HPV-positive cases, highlighting that optimized urine sampling alone cannot deliver perfect sensitivity. This discrepancy could stem from low viral loads, inconsistent shedding of exfoliated cells, or intrinsic constraints of urine as a diagnostic sample.

Among the various molecular assays assessed, PCR displayed higher HPV detection rates in this exploratory cohort (60%), followed by the line probe assay (46.7%), whereas Hybrid Capture-2 (20%) exhibited the lowest sensitivity in urine specimens. These observations are consistent with previous

studies reporting higher analytical sensitivity of PCR-based tests in samples with low viral load, such as urine [11, 12]. By comparison, HC-2, although commonly applied in cervical screening, demonstrated lower detection rates in urine samples, which may reflect reduced analytical sensitivity in low-DNA specimens [7, 11]. No HPV-negative participant displayed detectable urinary HPV DNA across any assay, suggesting that positive urine results are highly reliable, whereas negative results should be interpreted cautiously. PCR likely achieved the highest detection because target DNA amplification provides greater analytical sensitivity than the signal amplification used by HC-2, particularly in low-viral load urine. Although LPA covered more genotypes, its overall positivity remained lower than PCR.

Colposcopic and histopathological findings revealed a predominance of low-grade lesions (80%), with only one LSIL and one HSIL case identified. Although both cases were detected across all assays using Colli-Pee urine samples, this finding should be interpreted cautiously because only two lesions were detected, whereas testing of urine samples collected in disposable urine culture bottles failed to detect either case.

Genotype analysis of Colli-Pee collected urine samples of HPV-positive women showed that PCR and LPA identified overlapping yet distinct sets of HPV types. While PCR detected key high-risk HPV types (HPV16, HPV18, HPV31 and HPV35), LPA provided broader genotyping coverage, identifying additional high-risk (HPV33 and HPV45) and low-risk HPV types (HPV6, HPV44, HPV54, HPV61). These findings indicate that LPA yields more extensive genotyping data. The fact that some genotypes were exclusively detected by only one of the methods highlights the complementary strengths of these assays.

Urine-based sampling also demonstrated higher feasibility compared to cervical sampling, with a greater proportion of participants reporting ease of collection. This suggests that urine-based sampling may represent a feasible non-invasive alternative in settings where cervical sampling is less acceptable.

This exploratory study was limited by the small number of HPV-positive and CIN-positive women, which confined the statistical precision and limited the precise estimation of diagnostic accuracy. As this was an exploratory pilot study, the findings should be interpreted cautiously and validated in wider populations. Furthermore, genotyping was performed only on urinary samples and not on cervical samples, which precluded a direct comparison of genotype profiles between cervical and urinary specimens. This pilot study has several strengths, including the evaluation of cervical and urine samples from the same cohort and the comparison of multiple molecular assays. Moreover, the use of multiple diagnostic methods (HC-2, PCR, and LPA) enabled a broad comparison of test performance. An important methodological observation was that a standardized urine collection device can improve diagnostic yield, which is highly relevant for practical screening applications.

In conclusion, this pilot study conveys that standardized first-void urine collection using devices like Colli-Pee may improve urinary HPV DNA detection compared with routine urine collection methods. Even though urine-based HPV testing methods may serve as a feasible and non-invasive complementary approach, large studies employing standardized protocols are required to further establish their place in clinical practice.

Declarations

Ethical declaration and consent to participate: Ethical clearance was obtained from the Institutional Ethical Committee (IEC), All India Institute of Medical Sciences (AIIMS), New Delhi, India, with reference number: AIIMSA00216/13.12.2023, RT-07/18.01.2024. The study was conducted following approval by the IEC, which functions in accordance with ICH-GCP (International Council for Harmonisation - Good Clinical Practice), ICMR (Indian Council of Medical Research), and New Drugs and Clinical Guidelines-2019. Written informed consent was obtained from all study participants before enrolment.

Consent to publication: Not applicable.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Authors'contributions: **Conceptualization:** NS, **Methodology:** NS, GJD, PT, SM, AC **Software:** NS, GJD, SP **Validation:** NS, GJD, PT, PK, SP, AC, SM **Formal analysis and investigation:** NS, GJD, PT, PK, SP, SM, AC **Resources:** NS, NM, JM, SS, AS, AS, RK, **Data curation:** NS, GJD, TTK, SP **Writing- original draft preparation:** NS, TTK **Writing- review and editing:** NS, TTK, AG **Visualization:** NS, GJD, TTK **Supervision:** NS, JM, SS, RK, AS, AS, NM **Project administration:** NS, JM, SS, RK, AS, AS, NM **Funding acquisition:** NS

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Figures

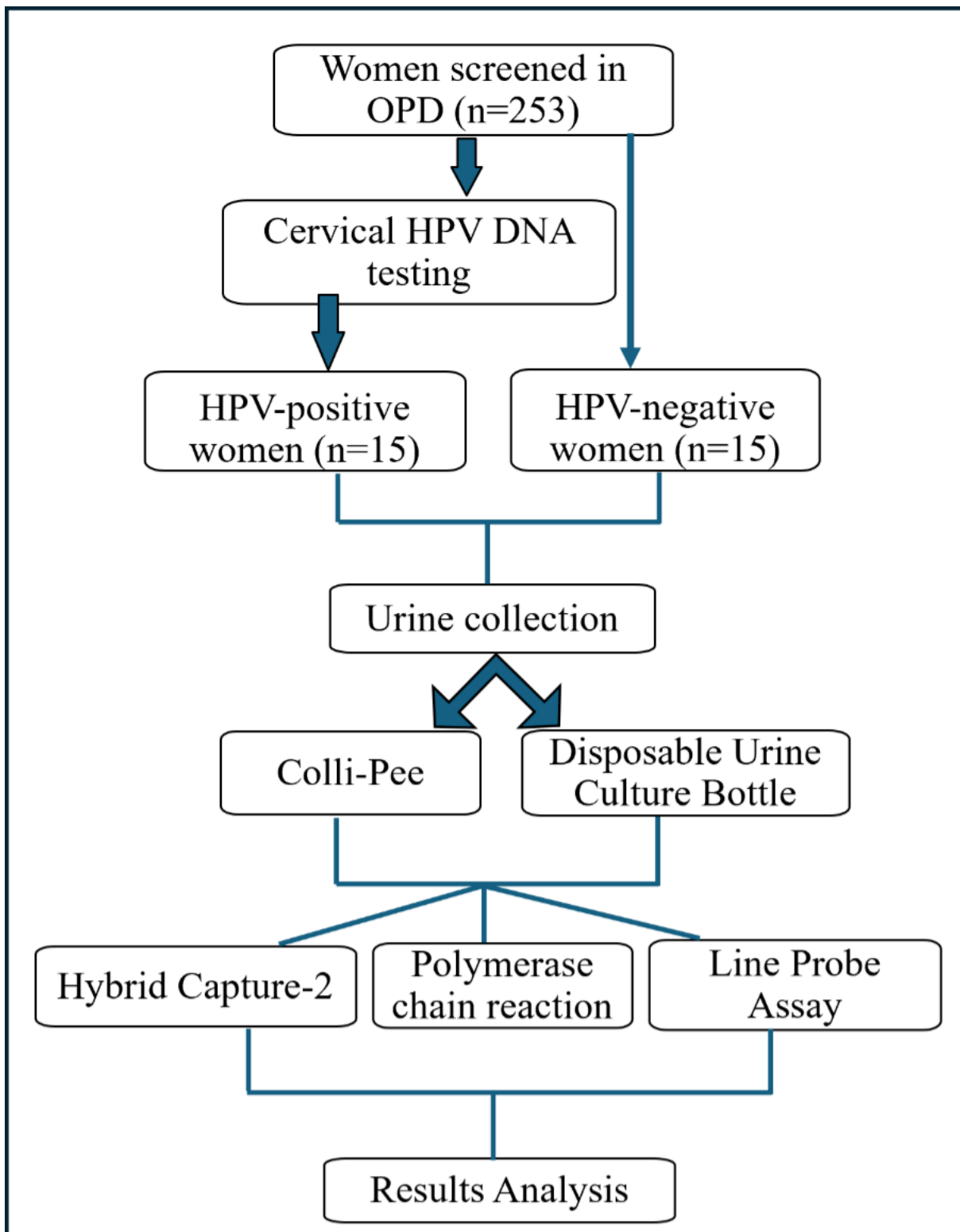


Figure 1

Study Schema

OPD- Outpatient Department

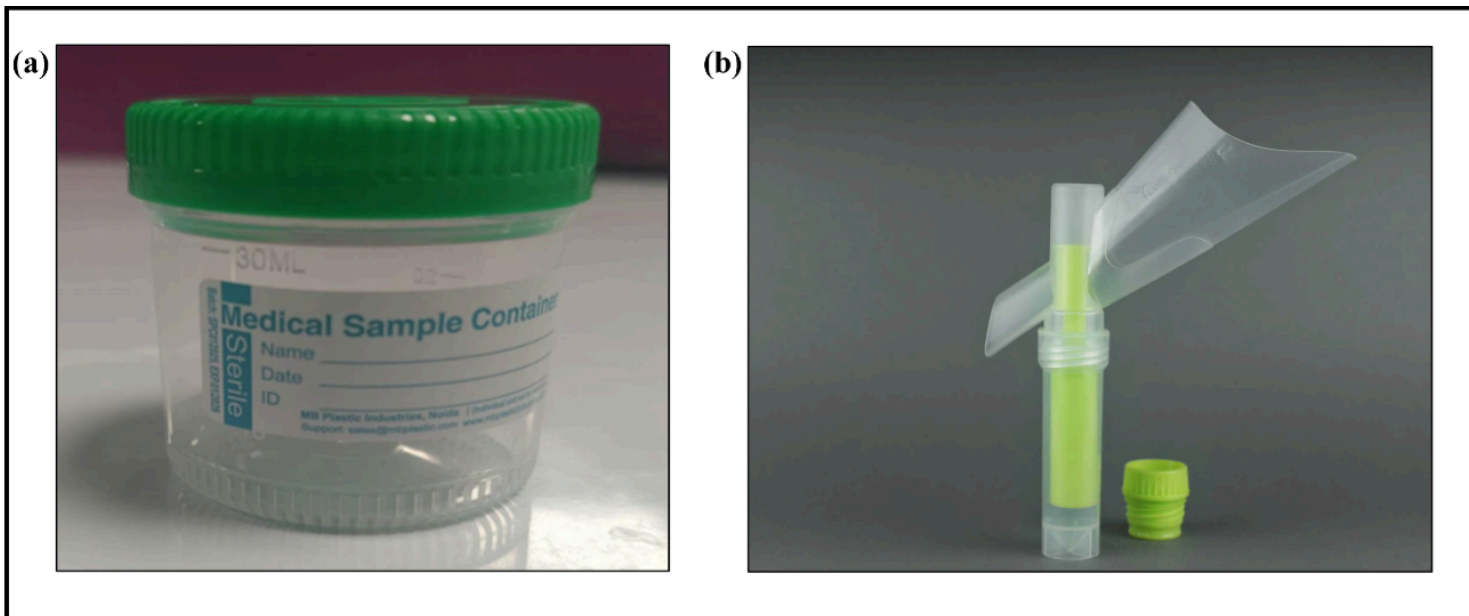


Figure 2

(a) and (b) – Urine sample collection methods used in the study.

(a) Disposable urine culture bottle is used for routine sample collection. (b) The Colli-Pee® device is designed for standardised first-void urine collection.

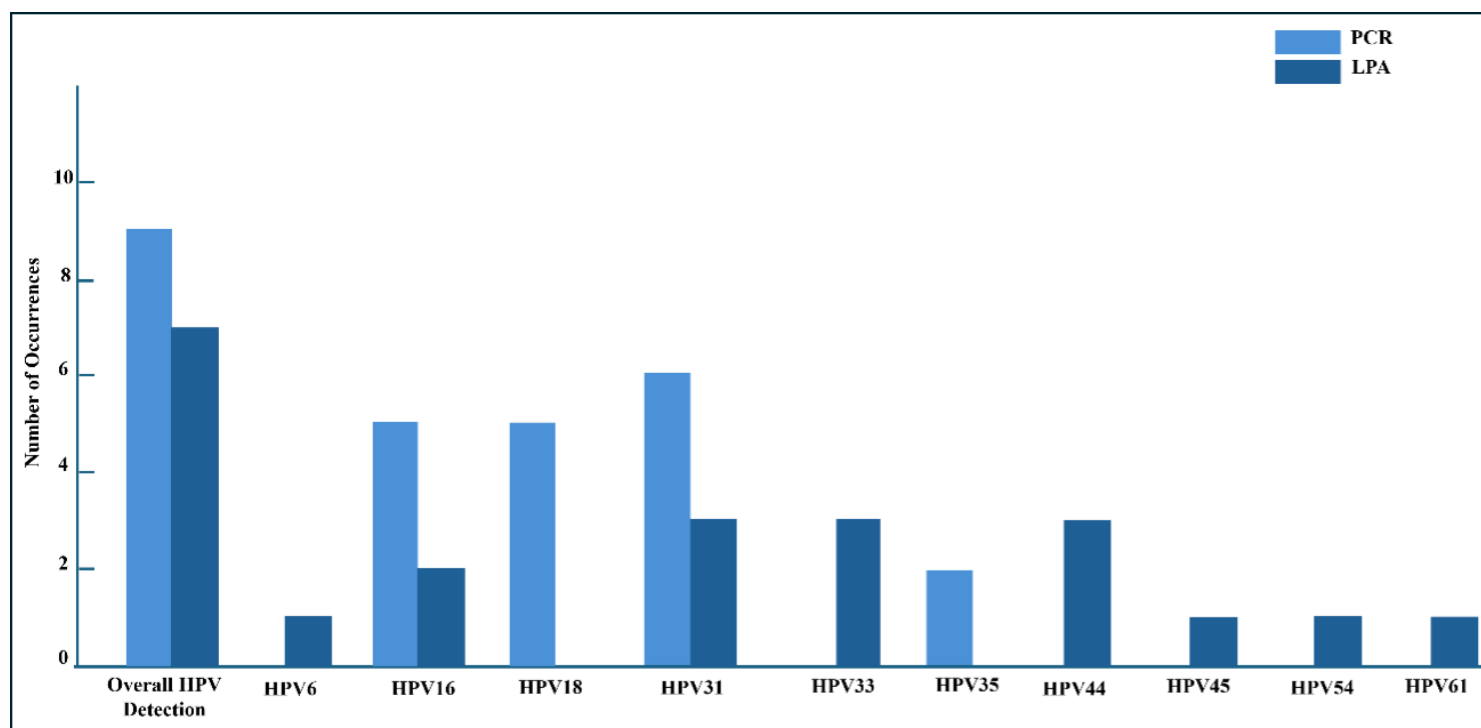


Figure 3

HPV genotype distribution (PCR versus LPA); HPV detection rates in Colli-Pee collected positive samples (n = 15). PCR detected more high-risk HPV genotypes (HPV16, HPV18, HPV31 and HPV35),

whereas LPA identified additional genotypes, (HPV6, HPV33, HPV44, HPV45, HPV54, and HPV61), indicating complementary detection patterns between the two assays. Individual participants may harbour more than one HPV genotype. Detection rates in Colli-Pee collected urine were 60% (9/15) by PCR, and 46.7% (7/15) by LPA, demonstrating that PCR has higher sensitivity when compared to other assays.

PCR = Polymerase Chain Reaction; LPA = Line Probe Assay

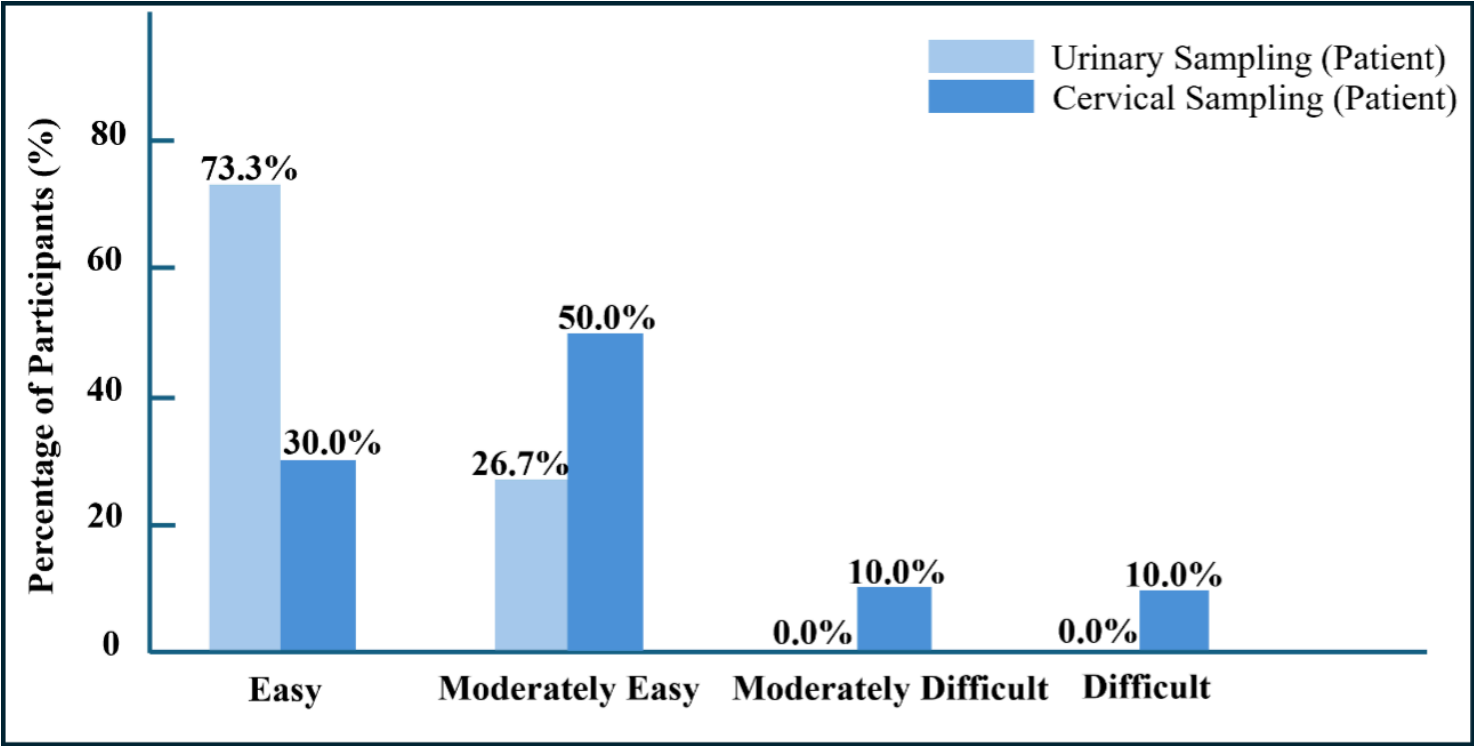


Figure 4

Feasibility of urinary versus cervical HPV DNA sampling on patient-reported experience (n = 30). A higher proportion of women rated the urine sampling method as easy and moderately easy (100.0%) compared with cervical sampling (80.0%), indicating greater feasibility of urine-based sampling.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementarymaterial.pptx](#)