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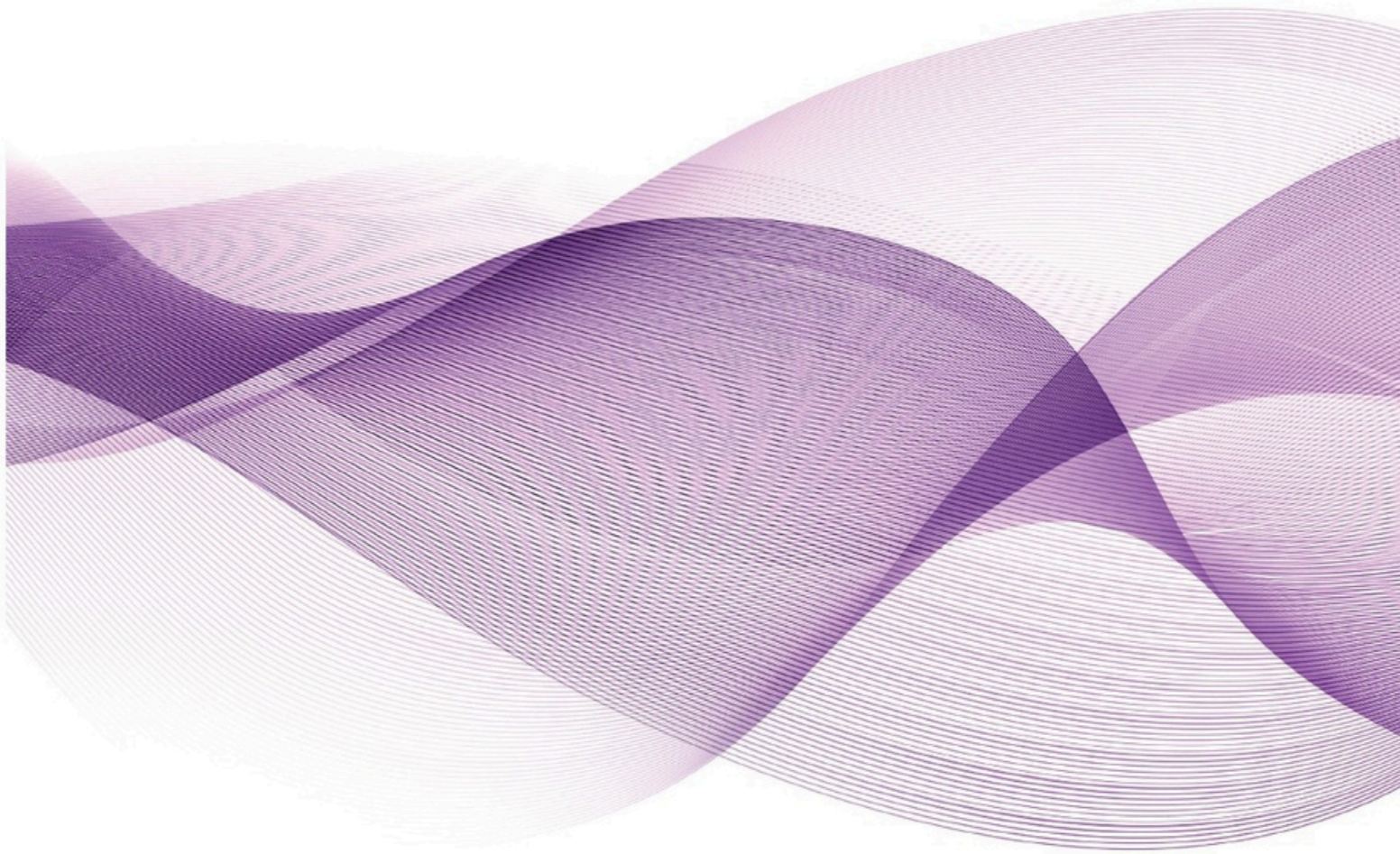

Supported by

梁愷昭婦癌基金會
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HPV Detection in Clinician-Collected, Self-Collected Vagina, Urine, and Menstrual Blood Samples

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Objective: This study evaluates the concordance, sensitivity, and specificity of self-collected samples—vaginal, urine, and menstrual blood—for detecting high-risk human papillomavirus (HrHPV) and high-grade squamous intraepithelial lesion (HSIL) compared with clinician-collected specimens for cervical cancer screening.

Methods: This prospective study was carried out in the colposcopy clinic of a tertiary hospital in Hong Kong. A consecutive series of premenopausal women referred to our colposcopy clinic due to abnormal Pap smears were screened and recruited. All participants provided self-collected vaginal, urine, and menstrual blood samples. Clinicians collected cervical samples for cytology and HPV testing. A colposcopy examination was performed if indicated. Testing for HrHPV in specimens was analyzed using a 14-type HPV-DNA test (Cobas 4800). Colposcopy with biopsy was used as the reference standard for histologic confirmation of cervical pathology.

Results: Of the 167 subjects included in the analysis, 85 (50.9%) had HrHPV infection. The overall concordance between clinician-collected HrHPV results and self-collected samples was 83.2% (vaginal), 79% (urine), and 82.1% (menstrual blood). Among the 167 cases, 139 underwent colposcopy and 42 cases (30.9%) of HSIL + were identified. The relative sensitivity for HSIL was 0.89 (95% CI, 0.79–1.02) for vaginal, 0.76 (95% CI, 0.63–0.91) for urine, and 0.83 (95% CI, 0.70–0.98) for menstrual blood samples in comparison to clinician-collected cervical samples.

Discussion: Self-collected vaginal, urine, and menstrual blood samples show moderate to good concordance with clinician-collected samples for HrHPV detection. However, only self-collected vaginal samples demonstrate comparable sensitivity and specificity for detecting HSIL.

Key Words: uterine cervical neoplasm, human papillomavirus viruses, early detection of cancer

(*J Low Genit Tract Dis* 2026;30:169–174)

Cervical cancer is caused almost exclusively by persistent infection with the human papillomavirus (HPV).[1] It is the fourth most common female cancer in the world. In 2022, an estimated 660,000 women were diagnosed with cervical cancer worldwide, and about 350,000 women died from the disease. The highest rates of incidence and mortality occurred in low- to middle-income countries, and this regional difference was attributed to inequalities in access to vaccination and health care facilities for screening and treatment services.

Globally, cervical cancer screening is shifting from cytology to high-risk human papillomavirus (HrHPV) testing.[2] Alongside this transition, sample collection techniques have advanced considerably. Notably, WHO guidelines now recognize self-collected vaginal samples for HrHPV detection as a viable alternative to clinician-collected samples. Meanwhile, emerging research suggests that urine and menstrual blood samples may also offer promising options for population-based screening programs.[3]

The primary aim of this study was to compare the concordance of self-collected vaginal, urine, and menstrual blood samples with clinician-collected cervical samples for HrHPV detection. Histologic CIN 2/3, corresponding to HSIL, was used as the disease endpoint. In addition, we evaluated and compared the diagnostic accuracy of each sampling method for detecting high-grade squamous intraepithelial lesions (HSILs) or worse, as determined by colposcopy-directed biopsy as the gold standard. To the best of our knowledge, this is the first study to assess and compare all 4 sampling methods simultaneously.

MATERIALS AND METHODS

Participants and Recruitment

This was a single-center, prospective cohort study of 167 premenopausal women who were referred to our colposcopy clinic because of an abnormal Papanicolaou smear result between December 2022 and September 2023. The participants were examined at the outpatient gynecology clinic of Queen Mary Hospital, which is a tertiary hospital in Hong Kong (Figure 1).

A priori sample size estimation was based on detecting a 10% difference in relative sensitivity between self-collected and clinician-collected samples for detecting high-grade squamous intraepithelial lesions (HSILs). Assuming a sensitivity of 90% for clinician-collected samples and an

Received for publication August 14, 2025; accepted March 8, 2026.
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Registration: University of Hong Kong (HKU)/Hong Kong West Cluster (HKWC) Institutional Review Board (IRB), registration number UW 22-607
Role of funding source: To fund consumables used for HPV testing, including test kits, assays, and machine consumables.
IRB status: Approved by the University of Hong Kong Institutional Review Board (IRB), registration number UW 22-607.
The authors declare that this evaluation was sponsored by Roche Diagnostics and the Karen Leung Foundation.
The authors have declared they have no conflicts of interest.
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DOI: 10.1097/LGT.0000000000000954

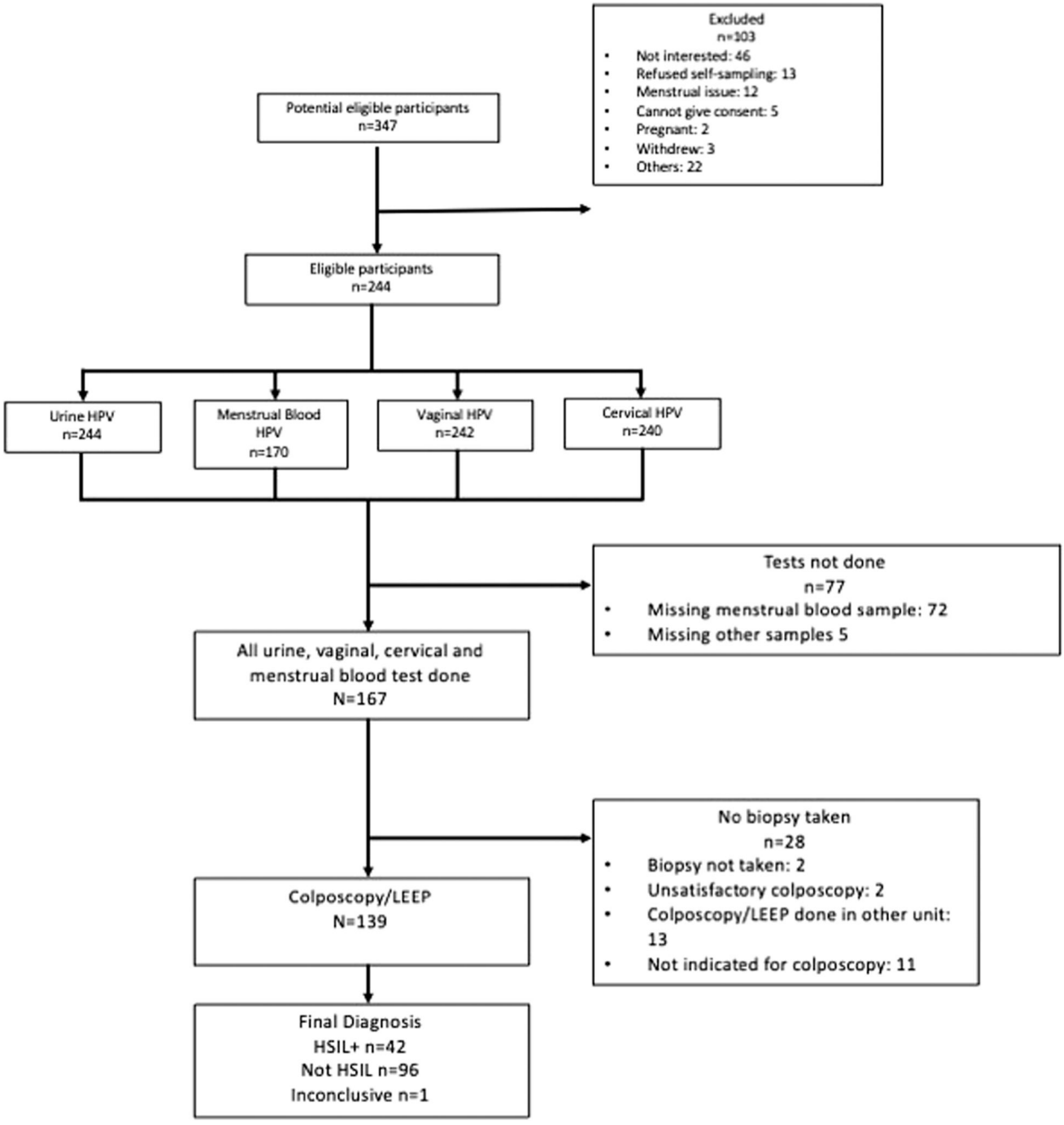

FIGURE 1. Flowchart for Recruitment

expected sensitivity of 85% for self-collected samples, with a 2-sided α of 0.05 and power (1–β) of 80%, a minimum of 180 paired samples would be required. This calculation follows methods described by Flahault et al and Jones et al for paired diagnostic test evaluation using McNemar's test, accounting for an estimated HSIL prevalence of 30% in the target population.[4]

The study was approved by the University of Hong Kong (HKU)/Hong Kong West Cluster (HKWC) Institutional Review Board (IRB), registration number UW 22-607. All participants provided informed consent before inclusion.

Eligibility Criteria

Inclusion Criteria. Eligible participants included those who were referred for an abnormal Pap smear based on the cytologic definitions of the 2014 Bethesda system. Participants had to be aged 18 years or older, with regular menstrual cycles (defined as cycle length not exceeding 3 mo), who were able to provide written consent and understand instructions for self-collection.

Exclusion Criteria. Women who were younger than 18 years, menopausal/perimenopausal (defined as cycle

TABLE 1. Pap Smear Results

Cytology result	N (%)
Unsatisfactory	2 (1.2)
Negative	62 (37.1)
AEC-NOS/AGC-NOS	5 (3.0)
ASC-US	56 (33.5)
LSIL	16 (9.6)
ASC-H	9 (5.4)
HSIL	14 (8.4)
SCC/adenocarcinoma	3 (1.8)

length exceeding 3 mo), pregnant, or had undergone a total hysterectomy, and those not willing to participate in the study were excluded.

Procedure

Participants were instructed to collect self-collected vaginal and urine samples before their clinical appointment. A pictorial guide and detailed explanation were provided by a study nurse to assist with the collection process. Each participant was asked to collect ~50 mL of first-pass urine in a standard sterile urine collection bottle. Fresh urine specimens were processed within 24 hours of collection to reduce DNA degradation. The urine samples were centrifuged at 1,800 rpm for 10 minutes. The solid pellet was resuspended in Thinprep PreservCyt solution (Hologic, Marlborough, MA), and the samples were stored at 4 °C before processing.

For the vaginal sample, participants used a thin plastic brush (FLOQSwabs, Copan Diagnostic, 2023, California), inserting it into the vagina and rotating it 10–30 times to gather cells. The collected vaginal specimens were then returned to the study nurse and placed in Thinprep PreservCyt solution.

The participant proceeded to have their doctor's consultation. During the clinic visit, a speculum was inserted into the vagina, and additional cervical cells were collected using a Cytobrush by the clinician. These specimens were also stored in Thinprep PreservCyt solution. Participants who were indicated for further examination underwent colposcopy and biopsy as necessary. After the clinic visit, participants were provided with a Q-pad (Qvin, Menlo Park, CA) for menstrual blood collection during their next menstrual cycle, which included a prepaid envelope for returning the sample to our laboratory for analysis. With the participant's consent, a total of 3 phone message reminders were sent to our patients in the following 3 months. All subjects were followed up according to our local college protocol (HKCOG 2024).[5]

Laboratory Testing

HPV Detection. All specimens were tested for HrHPV using the Cobas 4800 HPV test (Roche Molecular Diagnostics, Pleasanton, CA). The test specifically identifies HPV types 16 and 18, while concurrently detecting 12 other HrHPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). For all specimens, a 1 mL aliquot was loaded on the Cobas instrument, and all procedures were performed according to the manufacturer's instructions. Extraction for HPV-DNA on the menstrual pads was performed according to the manufacturer's instructions.

Liquid-Based Cytology. Cervical specimens were also sent for liquid-based cytology screening by the Department of Pathology at the University of Hong Kong, which is accredited by the College of American Pathologists (CAP). All slides were assessed by experienced cytotechnicians who were blinded to participant HPV and biopsy results, as well as to clinical examination findings throughout the study. Results were reported according to the 2014 Bethesda system.

Colposcopy-Directed Biopsy. Biopsies were performed during colposcopy when needed. Cervical biopsy specimens were stored in 10% buffered formalin bottles and embedded in paraffin. All biopsy reports were issued by pathologists from Queen Mary Hospital who were blinded to the study. Pathology results were classified into non-HSIL (i.e., negative, cervicitis, condyloma, CIN 1) and HSIL+ (i.e., CIN 2, CIN3, microinvasive or invasive squamous carcinoma, adenocarcinoma in situ, and adenocarcinoma).

Statistical Analysis

Concordance between clinician-collected cervical samples and self-collected vaginal, urine, and menstrual blood specimens was determined by calculating the overall, positive, and negative agreement and the kappa (κ) value, as per recommendations for evaluating a new test against a nonreference standard. Interpretation of the κ values followed the proposed standards of Landis and Koch: slight (0–0.20); fair (0.21–0.40); moderate (0.41–0.60); substantial (0.61–0.80); and almost perfect (0.81–1.00). In this study, clinician-collected samples were considered the nonreference standard, and self-collected samples were considered the tests. Diagnostic accuracy was defined as the proportion of correctly classified cases relative to the histopathologic reference standard (HSIL+ on colposcopy-directed biopsy) and was reported alongside sensitivity and specificity to facilitate comparison across tests. The diagnostic accuracy of these tests was also compared against colposcopy results, which serve as the reference standard for detecting HSIL. All CIs were at 95% confidence. A p value $< .05$ was considered statistically significant.

Subjects with any missing or inconclusive HPV or colposcopy biopsy result were excluded from the study. Patients who underwent a loop electrosurgical excision procedure (LEEP) before returning to menstrual pads were also excluded from the primary analysis.

TABLE 2. Concordance Calculations Against Clinician-Collected Cervical Specimens

Specimen	Number	Concordance (95% CI)	Positive agreement (95% CI)	Negative agreement (95% CI)	κ
Vagina	167	83.2% (77.6%–88.9%)	78.8% (70.1%–87.5%)	87.8% (80.7%–94.9%)	0.665
Urine	167	79.0% (72.9%–85.2%)	71.8% (62.2%–81.3%)	86.6% (79.2%–94.0%)	0.582
Menstrual blood (before LEEP)	156	82.1% (76.0%–88.1%)	72.4% (62.3%–82.4%)	91.3% (85.1%–97.4%)	0.639

TABLE 3. Sensitivity and Specificity for CIN 2+ Lesions

Specimen	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Accuracy (95% CI)
LBC (Clinician)	90.2% (81.2%–99.3%)	43.2% (33.2%–53.1%)	40.7% (30.6%–50.8%)	91.1% (82.8%–99.4%)	57.4% (49.0%–65.7%)
Cervical HPV (Clinician)	88.1% (78.3%–97.9%)	56.3% (46.3%–66.2%)	46.8% (35.8%–57.8%)	91.5% (84.4%–98.6%)	65.9% (58.0%–73.8%)
Vagina (Self-collected) HPV	78.6% (66.2%–91.0%)	59.4% (49.6%–69.2%)	45.8% (34.3%–57.3%)	86.4% (78.1%–94.6%)	65.2% (57.3%–73.2%)
Urine HPV	66.7% (52.4%–80.9%)	57.3% (47.4%–67.2%)	40.6% (29.0%–52.2%)	79.7% (70.2%–89.2%)	60.1% (52.0%–68.3%)
Menstrual pad HPV[a]	70.6% (55.3%–85.9%)	63.8% (54.1%–73.5%)	41.4% (28.7%–54.1%)	85.7% (77.5%–93.9%)	65.6% (57.4%–73.9%)

[a]Ten cases were omitted as a loop electrosurgical excision procedure (LEEP) was performed before the menstrual blood was sampled.

Data were stored using a secure Microsoft Excel file, and analysis was performed with SPSS.

RESULTS

The 167 participants ranged from age 21 to 53 years old, with a median age of 39. HrHPV was positive in 50.8% (85 out of 167) of clinician-collected samples. Eleven menstrual blood samples were excluded from primary analysis as a loop electrosurgical excision procedure (LEEP) was performed before the menstrual blood samples were saved. The cytology results obtained from participants are shown in Table 1.

Concordance with clinician-collected cervical samples for all HrHPV was highest in the vaginal group at 83.2% (139 out of 167 cases, 95% CI: 77.6%–88.9%, $\kappa = 0.665$), followed by menstrual blood at 82.1% (128 out of 156 cases, 95% CI: 76.0%–88.1%, $\kappa = 0.639$) and urine at 79.0% (132 out of 167 cases, 95% CI: 72.9%–85.2%, $\kappa = 0.582$). They all showed moderate to substantial agreement (Table 2).

One hundred thirty-nine out of 167 cases underwent colposcopy, with biopsy done. As shown in Table 3, the rate of biopsy-proven HSIL+ lesions was 30.9% (42 out of 138 cases); ASC-US and above was used as the threshold for liquid-based cytology (LBC). The sensitivity for high-grade lesions was highest in the LBC group at 90.2% (37 out of 41 cases, 95% CI: 81.2%–99.3%), followed by clinician-collected cervical at 88.1% (37 out of 42 cases, 95% CI: 78.3%–97.9%), self-collected vaginal at 78.6% (33 out of 42 cases, 95% CI: 66.2%–91.0%), menstrual pad at 70.6% (24 out of 34 cases, 95% CI: 55.3%–85.9%), and urine at 66.7% (28 out of 42 cases, 95% CI: 66.2%–91.0%). Specificity, on the other hand, was highest in the menstrual pad group at 63.8% (60 out of 94 cases, 95% CI: 54.1%–73.5%), followed by self-collected vagina at 59.4% (57 out of 96 cases, 95% CI: 49.6%–69.2%), urine at 57.3% (55 out of 96 cases, 95% CI: 47.4%–67.2%), clinician-collected cervical at 56.3% (54 out of 96 cases, 95% CI: 46.3%–66.2%), and LBC at 43.2% (41 out of 95 cases, 95% CI: 33.2%–53.1%).

Table 4 summarizes the relative sensitivity and specificity of various tests for detecting HSIL+ against clinician-collected LBC as the reference standard. Self-collected vaginal samples exhibited a relative sensitivity of 0.86 (95% CI: 0.70–1.06, $p = .267$). Urine samples showed a relative sensitivity of 0.76 (95% CI: 0.61–0.94, $p = .022$). Menstrual blood samples had a relative sensitivity of 0.80 (95% CI: 0.61–1.00, $p = .180$). Based on these results, vaginal and menstrual blood samples showed no statistically significant difference in relative sensitivity compared with clinician-collected smears. In terms of specificity, all samples had a statistically significant improved specificity. Self-collected vaginal samples had a relative specificity of 1.37 (95% CI: 1.03–1.81, $p = .040$), while urine samples had a relative specificity of 1.32 (95% CI: 0.97–1.78, $p = .098$). Menstrual blood samples demonstrated a relative specificity of 1.49 (95% CI: 1.13–1.95, $p = .006$).

Table 5 presents the relative sensitivity and specificity of self-collected samples compared with clinician-collected cervical HPV testing for detecting HSIL+ lesions. Self-collected vaginal samples recorded a relative sensitivity of 0.89 (95% CI: 0.78–1.02, $p = .219$), showing no statistically significant difference from the clinician-collected HPV test. On the other hand, urine samples and menstrual blood samples demonstrated a relative sensitivity of 0.76 (95% CI: 0.63–0.91, $p = .004$) and 0.73 (95% CI: 0.60–0.89, $p = .002$), respectively, showing a significant difference when compared with the clinician-collected HPV test. Regarding

TABLE 4. Relative Sensitivity and Specificity for Detecting CIN 2+ Lesions Against Liquid-Based Cytology (LBC)

Test	Relative sensitivity (95% CI)	*p*	Relative specificity (95% CI)	*p*
LBC (Clinician)	1		1	
Cervical HPV (Clinician)	0.97 (0.83–1.14)	1.000	1.29 (0.98–1.71)	.096
Vaginal HPV (Self)	0.86 (0.70–1.06)	.267	1.37 (1.03–1.81)	.040
Urine HPV	0.76 (0.61–0.94)	.022	1.32 (0.97–1.78)	.098
Menstrual blood HPV (before treatment)	0.80 (0.61–1.00)	.180	1.49 (1.13–1.95)	.006

specificity, self-collected vaginal samples had a relative specificity of 1.06 (95% CI: 0.90–1.24, $p = .664$), while urine samples maintained a relative specificity of 1.02 (95% CI: 0.86–1.21, $p = 1.000$). Menstrual blood samples exhibited a relative specificity of 1.15 (95% CI: 0.98–1.35, $p = .134$).

DISCUSSION

HPV self-sampling has been approved by various international organizations, such as the Food and Drug Administration (FDA) and the WHO, as a valid alternative to clinician-collected HPV. In this study, the concordance between clinician-collected and self-collected vaginal samples was 83.2% (95% CI: 77.6%–88.9%, $\kappa = 0.665$). This is consistent with findings from Arbyn et al.[6] for self-collected vaginal samples using validated PCR methods. Local studies by Ngu et al.[7] reported similar concordance rates, emphasizing the reliability of self-collection.

Few large-scale studies have reported the efficacy of HPV detection in menstrual blood. Our study reports a concordance of 82.1% (95% CI: 76.0%–88.1%, $\kappa = 0.639$), which shows substantial agreement. However, it is still lower than those quoted by Naseri et al.[8] and Zhang et al.,[9] who reported concordance rates between 92.7% and 94%, which is even higher than that of self-collected vaginal samples. Our study excluded patients who had an LEEP procedure (11 patients) before menstrual blood collection. Upon analysis of these 11 cases, the concordance and sensitivity rate after LEEP were significantly lower at 45.5% (95% CI: 16%–74.9%, $\kappa = 0.154$) and 37.5% (95% CI: 4%–71%), which is in line with studies evaluating HPV persistence after LEEP.[10] Furthermore, 167 out of 200 menstrual pads (83.5%) were returned to our laboratory through the local mail, which demonstrates a relatively high participation rate when compared to local cervical cancer screening rates. Notably, up to 3 reminders were sent in this study, which may be more frequent than real-life practice.

The first meta-analysis on urine HPV testing by Pathak et al.[11] was published in 2014 and concluded that urine HPV testing was a feasible and acceptable alternative in cervical cancer screening. It quoted a pooled sensitivity of 77% for HrHPV when using PCR-based techniques, which is similar to that found in our study. One limitation of this study is that recent studies have shown superior sensitivity for HSIL with the use of a new device, where a fixed first-void urine (FVU) was used instead of a standard collection pot. The urine HPV test sensitivity for HSIL+ was higher with the FVU-collection device (90.3%, 95% CI: 83.7%–94.9%, 112/124) than the standard pot (73.4%, 95% CI: 64.7%–80.9%, 91/124, $p = .0005$).[12] Unfortunately, this device was not available during the commencement of this study, and thus, results from this study might not truly reflect the latest standards to which urine HPV testing has reached.

Histopathology on colposcopy-directed biopsy, which is considered the diagnostic reference standard, allows us to compare the relative sensitivity and specificity for detecting HSIL. Compared to conventional cytology, only self-collected vagina samples showed similar relative sensitivity of 0.86 (95% CI: 0.78–1.02, $p = .267$). Whereas menstrual pad and urine samples were significantly less sensitive, with relative sensitivity of 0.73 (95% CI: 0.60–0.89, $p = .002$) and 0.76 (95% CI: 0.63–0.91, $p = .004$), respectively. On the other hand, all tests showed significantly improved relative specificity compared to conventional cytology. When compared to clinician-collected HPV as the standard test, only self-collected vaginal samples showed comparable relative sensitivity at 0.89 (95% CI: 0.78–1.02, $p = .219$). They all demonstrated similar relative specificity.

Limitations

The study has several important limitations that should be acknowledged. First, this study did not reach its planned recruitment target, and the primary comparisons are therefore underpowered; all effect estimates should be interpreted as imprecise and hypothesis-generating rather than confirmatory. Second, the cohort consisted of women referred to a colposcopy clinic with abnormal cytology, representing a referral population from a single tertiary center in Hong Kong, which may limit generalizability to routine screening settings or other health systems. The sensitivity of traditional cytology for detecting cervical intraepithelial neoplasia (CIN) is typically reported as 50%–75%, yet our participants were already at increased risk because of abnormal Papanicolaou smears; this enrichment likely inflates the observed cytology sensitivity, which may not be directly applicable to unselected screening populations.[13] Third, verification bias is possible because not all participants underwent colposcopy with biopsy, and histologic confirmation was more likely among women at higher clinical risk, which may have influenced estimates of sensitivity, specificity, and diagnostic accuracy. Taken together, these factors mean that the results should be interpreted cautiously, as hypothesis-generating rather

TABLE 5. Relative Sensitivity and Specificity for CIN 2+ Lesions Against Clinician-Collected HPV

Test	Relative sensitivity (95% CI)	*p*	Relative specificity (95% CI)	*p*
Cervical HPV (Clinician)	1		1	
Vaginal (Self)	0.89 (0.78–1.02)	.219	1.06 (0.90–1.24)	.664
Urine	0.76 (0.63–0.91)	.004	1.02 (0.86–1.21)	1.000
Menstrual blood (before treatment)	0.73 (0.60–0.89)	.002	1.15 (0.98–1.35)	.134

than definitive, and future larger, adequately powered multicenter studies are needed to confirm the diagnostic performance of these self-collected sampling methods.

CONCLUSIONS

This single-center exploratory study suggests that self-collected vaginal, urine, and menstrual blood samples may offer acceptable concordance with clinician-collected specimens for HrHPV detection, with self-collected vaginal samples showing the most promising performance for HSIL+ detection. These findings should be viewed as hypothesis-generating in light of the limited sample size and underpowered comparisons, and larger, adequately powered multicenter studies are needed before urine and menstrual blood collection can be recommended for routine cervical cancer screening.

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