Gynecologic Oncology Reports 54 (2024) 101421

Contents lists available at ScienceDirect

Gynecologic Oncology Reports

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

Variables that impact HPV test accuracy during vaginal self collection workflow for cervical cancer screening

Laurence Vaughan [a,1,*], Devin Gary [a], Millie Shah [b], Lyndsay Lewellen [b], Laura Galbraith [b], Valentin Parvu [a]

[a] Scientific Affairs, Becton, Dickinson and Company, BD Life Sciences – Diagnostic Solutions, 7 Loveton Circle, Sparks, MD 21152, USA
[b] Research and Development, Becton, Dickinson and Company, BD Life Sciences – Diagnostic Solutions, 54 Loveton Circle, Sparks, MD 21152, USA

ARTICLE INFO

Keywords:
HPV self collection
Uterine cervical neoplasms
Specimen handling
Analytic sample preparation methods
Human papillomavirus viruses
Clinical laboratory techniques

ABSTRACT

Vaginal self collection (SC) is safe and effective for human papillomavirus (HPV) testing and can increase cervical cancer screening coverage for underserved women. To better understand the impact of SC methodology on HPV test outcomes, empirical testing was conducted using different swab collection workflows. Deposition of the collection swab into resuspension buffer resulted in a 2.4-cycle reduction in threshold detection of human beta-hemoglobin during PCR when compared to "swirl-and-toss". In addition, reducing the swab resuspension volume from 10 mL to 3 mL resulted in a 2.6-cycle reduction in threshold detection of human beta-globin. A systematic literature search (01/01/2020 to 08/02/2023) of Ovid Medline and Embase, followed by data extraction and analysis, was conducted to further assess the impact of resuspension volume on performance following SC. HPV test performance for SC, relative to clinician collection (CC), was calculated for detection of cervical pre-cancer. Data were stratified by the resuspension volume ratio of SC to CC being either ≥ 1.0 or < 1.0. SC with a volume ratio of ≥ 1.0 and < 1.0 had a relative $\geq$ CIN2 sensitivity of 92.0 % (95 % CI: 88.0, 96.0) and 97.0 % (95 % CI: 94.0, 100), respectively. Taken together, these results suggest that SC conditions can be modified to optimize sample recovery and performance, as part of cervical cancer screening.

1. Introduction

Reaching under- or never-served women—those not attending routine screening even if it is provided at no or low cost by the health service—remains a major challenge for global cervical cancer screening programs (Bruni et al., 2022). In the United States, 60 % of cervical cancer is diagnosed in women who do not undergo routine screening (about 30 % of all screen-eligible women) (Benard et al., 2021). Thus, it is critical to expand screening coverage for detection and treatment of pre-cancer in under- or never-screened women. In recent years, self collection (SC) has emerged as the preferred solution that enables women to collect vaginal samples at home or in any clinic setting to offset barriers including privacy concerns and poor healthcare access (Arbyn et al., 2022; Serrano et al., 2022). Meta-analyses have shown that DNA-based PCR detection of SC vaginal samples is non-inferior to in-office clinician-collected (CC) endocervical specimens, and that it can significantly increase uptake in national screening programs (Arbyn et al., 2022; Arbyn et al., 2018).

Arbyn et al have proposed VALHUDES (a protocol for **VAL**idation of **HU**man papillomavirus assays and collection **DE**vices for HPV testing on **S**elf-samples and urine samples) as an approach to validate the performance of specific assay and device combinations (Arbyn et al., 2018; De Pauw et al., 2021). This protocol is modeled on the now well-established Meijer criteria for validation of primary screening assays and VALGENT protocols for validation of genotyping assay performance (Arbyn et al., 2018; Meijer et al., 2009). The VALHUDES protocol is increasingly being used to validate specific assays and devices, several of which have been demonstrated as non-inferior in sensitivity and specificity to that from paired CC samples (Latsuzbaia et al., 2022; Van Keer et al., 2022; Latsuzbaia et al., 2022; Latsuzbaia et al., 2023).

Although the performance of HPV assays following SC has been shown to be comparable to HPV assay performance following CC of endocervical specimens, some studies do show considerable differences in performance, which may correspond to deviations between SC

* Corresponding author at: Medical and Scientific Affairs, Becton, Dickinson and Company, BD Life Sciences – Diagnostic Solutions, 7 Loveton Circle, Sparks, MD 21152, USA.
E-mail address: laurence.vaughan@bd.com (L. Vaughan).
[1] To whom inquiries regarding the paper should be directed.

https://doi.org/10.1016/j.gore.2024.101421
Received 10 April 2024; Received in revised form 14 May 2024; Accepted 17 May 2024
Available online 25 May 2024

workflows. Previous work has identified factors that could negatively impact performance following SC. For example, Cadman et al reported differential performance using the same reference assay and experimental protocol with different collection devices in a controlled setting, with two devices performing less consistently than others (Cadman et al., 2021). Arbyn and colleagues (2022 and 2023) also confirmed reduced performance of one of those devices using the VALHUDES protocol (Latsuzbaia et al., 2022; Latsuzbaia et al., 2023). The performance of the BD Onclarity assay, following SC, has recently been evaluated using the VALHUDES protocol, resuspending swabs in 3 mL HPV diluent, and showed non-inferior sensitivity to CC samples (Martinelli et al., 2024).

Many studies have used liquid-based cytology (LBC) media to resuspend SC specimens because it has been the gold standard media for both cytology and HPV testing for over two decades (Latsuzbaia et al., 2022; Polman et al., 2019; Inturrisi et al., 2021). For practicality, it is reasonable to compare workflows using LBC, with the exception that one sample is collected from the vagina (SC) and the other from the endocervix (CC). However, LBC samples use either 10 mL of BD SurePath™ Liquid-based Pap Test ("SurePath"; Becton, Dickinson and Company; BD Life Sciences—Diagnostics Solutions, Sparks, MD) or 20 mL of ThinPrep® Pap Test PreservCyt® Solution ("PreservCyt;" Hologic, Inc.; San Diego, CA, USA) resuspension volume for Pap smear collection and, thus, some have used these standard volumes for SC devices (Latsuzbaia et al., 2022; Tranberg et al., 2018). The 10 to 20 mL volume is designed for cytology testing, which typically requires 8 mL (SurePath) or 5–15 mL (PreservCyt) of media, leaving the residual volume for HPV or other analyte testing. LBC is designed to fix cells and conserve them for cytologic analysis. The goal is to deposit cells in a thin layer on a glass slide in a controlled fashion, which requires a larger resuspension volume than is typically required for molecular testing (2–3 mL on average). Thus, from a technical standpoint, LBC is not particularly well suited to molecular analysis because it dilutes the analyte. While there are adequate numbers of cells available for sampling in the vagina compared to the endocervix, high-risk HPV infections have a particular tropism for cells of the endocervix (specifically the rapidly dividing cells of the transformation zone), which can result in higher viral loads when comparing the endocervix and vaginal papillomavirus microbiome (Li et al., 2023; de Sanjose et al., 2017). Thus, it is critically important to have optimal workflows for vaginal SC to ensure optimal sensitivity and non-inferior performance compared to gold standard, CC endocervical samples. Here, a combination of empirical sample testing and literature review was employed to determine the degree that resuspension method and resuspension volume impact specimen recovery and HPV assay performance.

2. Methods and materials

2.1. Empirical sample testing

2.1.1. Sample collection and swab resuspension

Empirical testing was performed to identify variables in a SC workflow that may impact analytical performance. The Cycle threshold (Ct) score for the human beta-globin (HBB) target, corresponding to the internal control primer set of the BD Onclarity™ HPV Assay ("Onclarity;" Becton, Dickinson and Company; BD Life Sciences—Diagnostics Solutions, Sparks, MD), was used to assess sample recovery. Three experimental setups (Table S1) were utilized to differentiate the impact on HBB detection by altering either collection method (SC versus CC), media type (SurePath versus PreservCyt, etc.), collection volume, and resuspension method (swab deposit versus swirl-and-toss). Individual SC vaginal samples (SC swabs) were obtained from healthy donors as part of an in-house open enrollment sample collection program, approved under central IRB (Advarra). SC swabs (SC swabs) were collected using Copan FLOQSwabs® ("FLOQSwab;" Copan Italia S.p.a.) per manufacturer instructions. SC swabs were stored at −20 °C before use and were tested within 90 days (prior experiments established that storage at −20 °C had no impact on sample integrity) (BD internal data on file). SC swabs were thawed for approximately 30 min prior to use.

2.1.1.1. Experiment 1. For the swirl-and-toss experimental condition, one SC swab per donor was placed in each BD SurePath vial (10 mL) ("SurePath;" Becton, Dickinson and Company; BD Life Sciences—Diagnostic Solutions; Sparks, MD) and expressed by fully immersing the tip of the SC swab in the large opening of the vial and swirling for 10 s. Excess liquid from the SC swab was removed by lightly wiping the SC swab against the edge of the large opening of the vial prior to discarding the SC swab. For the swab deposit experimental condition, one SC swab per donor was expressed into each SurePath vial (10 mL) by cutting the stem of the SC swab at about 30 mm and depositing it in the large opening of the vial.

2.1.1.2. Experiment 2. SC was performed using one FLOQSwab per person, with swabs resuspended at 1 swab per 1 mL of sterile saline to generate a vaginal matrix. FLOQSwabs were then dipped in the matrix, allowed to dry, and then either deposited directly in 3 mL of HPV diluent buffer ("HPV DB;" a lytic preservative media) or swirled in 5 mL of PreservCyt media and then discarded (swirl-and-toss), prior to HPV assay testing. For the conditions involving SC swab deposition in HPV DB, FLOQSwabs were broken off at the 60 mm score mark and deposited directly into tubes containing 3 mL HPV DB.

2.1.1.3. Experiment 3. The SC swab preparation method (10 mL of SurePath with swirl-and-toss; see Experiment 1) was also utilized as part of Experiment 3. Specimens involving deposition of SC (as in

Experiment 2) FLOQSwabs, directly into 3 mL HPV DB, were also utilized in Experiment 3. CC standard of care reference specimens were collected as part of a US national clinical trial where either brush/spatula or cervex brushes were broken off and deposited into 10 mL SurePath vial compartment prior to transport to the laboratory for testing (Stoler et al., 2018).

2.1.2. HPV testing

All contrived and clinical specimens were tested using Onclarity on either the FDA-approved mid-volume BD Viper™ LT (Becton, Dickinson and Company; BD Life Sciences—Diagnostic Solutions; Sparks, MD) or the high-volume BD COR™ systems (Becton, Dickinson and Company; BD Life Sciences—Diagnostic Solutions; Sparks, MD), in accordance with manufacturer instructions. Both platforms are fully integrated sample preparation and analytic testing systems that extract target DNA from patient specimens and perform real-time PCR detection (Young et al., 2020). Onclarity has been described previously; (Bottari and Iacobone, 2019) briefly, it is a 15-target (14 HPV genotypes and 1 internal cellularity control), multiplex, real-time PCR assay approved for cervical cancer screening. Ct scores generated by the beta-globin internal (cellularity) control were used to estimate cell recovery. When comparing different recovery methods, a 1 Ct score reduction was assumed to represent an approximate 2-fold increase in recovered cells. Standard statistical methods were used to generate mean Ct scores and confidence intervals (Minitab®).

2.2. Literature review

The methodologies for research and reporting of systematic literature review or *meta*-analysis results, outlined by the Cochrane Collaboration Diagnostic Test Accuracy Working Group and by Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines, were followed during literature searching, article review, and data extraction for this study. A literature search was conducted that identified eligible sources between 01/01/2020 to 08/02/2023 in order to identify articles published subsequent to the *meta*-analysis publication

by Arbyn et al., 2022 (which provides a comprehensive analysis of HPV assay performance following SC as compared to CC) (Arbyn et al., 2022); and that were relevant to the following PICOs: P = women undergoing HPV testing for screening, follow-up, or post-treatment; I = performance of SC; C = performance of CC; O = relative performance of SC compared to CC with either CC results (positive and/or negative percent agreement to HPV results) or high-grade cervical disease (sensitivity and/or specificity for disease detection) as the reference. Titles were obtained through the Ovid platform to query both Medline and Embase.

A search string was constructed (Table S2) to capture articles identified in the fields of cervical cancer screening, human papillomavirus, and SC specimens. In total, 179 titles were identified through Ovid (Medline and Embase, combined) (Figure S1). In addition, 12 references were identified through hand searching, which contained results relevant to both performance and other aspects of the SC workflow; these references included performance based on device storage and resuspension volume, performance of SC across different HPV assays, impact on screening/follow-up attendance following SC, and long-term safety of SC during cervical cancer screening. Exclusion criteria were: (1) Title/source from a non-credible source; (2) Title/source contained an ambiguous research question; (3) No performance data specific to HPV assays from SC or CC; (4) Did not identify/involve HPV testing or cervical cancer screening; (5) No clear reference/comparator; (6) Data were collected in an unethical manner; (7) The index test involved a mechanism other than RT-PCR for HPV detection; (8) Data could not be extracted for analysis; and (9) No data regarding sensitivity or specificity for the index test relative to the reference/comparator test. Additional secondary exclusion criteria included title/source not in the English language; and the study did not involve humans. Remaining articles were required to have methodological information and outcome results (e.g., collection device, LBC type, transport conditions, storage volume) sufficient to perform calculations across studies. After abstract/title and full text screening, 17 articles remained in the analysis; 10 articles (Latsuzbaia et al., 2022; Latsuzbaia et al., 2022; Cho et al., 2021; El-Zein et al., 2018; Castle et al., 2020; Cocuzza and Arbyn, 2021; Ertik et al., 2021; Rohner et al., 2020; Rohner et al., 2020; Stanczuk et al., 2022) were utilized for *meta*-analysis and the remaining 7 articles (Polman et al., 2019; Terada et al., 2022,; Eamratsameekool et al., 2023; Esber et al., 2018; Ilardo et al., 2022; Onuma et al., 2020; Martinelli et al., 2022) were included as part of supplemental analysis.

Six domains were utilized to ascertain the overall quality and strength of each, included reference: (1) consistency, (2) directness, (3) precision, (4) bias, (5) magnitude of effect, and (6) confounder effect (Table S3). Scoring for the six domains utilized during quality assessment consisted of poor (0 points), moderate (0.5 points), and good (1 point). The overall quality ranking was determined by the average of the six scores, for which 0 to ≤ 0.33 resulted in a poor overall rank, >0.33 to ≤ 0.67 resulted in a moderate overall rank, and > 0.67 to 1 resulted in a good overall rank (Table S4).

2.3. Data analysis

Data extraction was facilitated by two reviewers/authors with any results discrepancies adjudicated by a third reviewer/author; an independent author performed all statistical methods. Analyses were conducted using R software (version 4.0.2) with the meta and metafor packages. For each study, the ratios of SC vs. CC specimens were calculated along with a 95 % confidence interval. Log-transformed ratios were combined to obtain random effects estimates regarding overall relative sensitivity and relative specificity of SC vs CC specimens, using inverse variance weighting. *Q-tests* for heterogeneity based on random effects models were used to evaluate statistical differences between subgroups. Funnel plots of relative sensitivity and relative specificity were generated for all studies combined.

3. Results

3.1. Empirical testing

The impact of resuspension method on threshold detection of HBB was assessed using empirical testing of pooled vaginal matrices to create replicate SC samples. FLOQSwabs were dipped into a common pooled vaginal matrix and allowed to air-dry overnight to simulate vaginal dry SC specimens. First, the impact of (1) depositing the SC swab in 10 mL SurePath preservative media (n = 15) versus (2) swirling the device head in the solution and then discarding it (swirl-and-toss; n = 15) was investigated. Deposition of the SC swab led to an approximate 1 Ct earlier score compared to swirl-and-toss; suggesting an approximately 2-fold difference in cell recovery (or 50 % loss in potential target acquisition with swirl-and-toss; Fig. 1A).

A published method was compared for recovery of SC swabs in (1) 5 mL PreservCyt media, swirling the SC swab for 20 s before discarding it (n = 30), versus (2) breaking off the SC swab head using a score mark on the shaft and depositing in 3 mL of lytic preservative media (HPV DB; n = 29; originally n = 30, however, there was one invalid run). The 3 mL HPV direct deposit recovery method exhibited a mean Ct value that was 4 cycles earlier than SC swab swirl-and-toss in 5 mL PreservCyt media, corresponding to an approximate 16-fold higher target recovery (Fig. 1B).

Finally, target recovery was compared using two different resuspension methods in 10 mL of SurePath media (Bruni et al., 2022) swirling the dry SC swab into 10 mL SurePath [n = 97] versus (Benard et al., 2021) CC standard of care [n = 33,623; where the entire cervical device is deposited into 10 mL SurePath]) and versus SC swab recovery in 3 mL HPV diluent (n = 57). A 2.4 times lower mean Ct value in CC versus the 10 mL SC swirl-and-toss method and an additional 2.6 times lower Ct using the 3 mL direct deposit SC method compared to the CC standard of care (Fig. 1C).

3.2. Literature review

Recent literature relevant to SC for cervical cancer screening was examined and 17 studies (Figure S1) were identified in which SC performance, relative to CC, was reported. Variables of interest included SC sample resuspension volume and transport condition. Unfortunately, no studies thoroughly described the swab deposition method in a manner that facilitated data retrieval and analysis for this study and so comparison could not be performed through literature review for the resuspension method. Ten (10) of the studies reported SC performance relative to a reference method, ≥CIN2; they were utilized for *meta*-analysis (Table 1; Figs. 2 and 3). (Latsuzbaia et al., 2022; Latsuzbaia et al., 2022; Cho et al., 2021; El-Zein et al., 2018; Castle et al., 2020; Cocuzza and Arbyn, 2021; Ertik et al., 2021; Rohner et al., 2020; Rohner et al., 2020; Stanczuk et al., 2022) In order to compare the relative performance of SC to CC for detection of cervical disease cases by resuspension volume, data were divided into two groups: (1) SC volume-to-CC volume ratio = ≥1.0, and (2) SC volume-to-CC volume ratio < 1.0. The quality of evidence was good for most included articles, with some articles judged as moderate in quality (Table S3). In addition, funnel plot analyses showed no significant asymmetry, indicating minimal publication bias (p-values for Egger's test were both > 0.05 in Figure S2 and Figure S3). When stratified by SC: CC volume ratio of ≥ 1,0 and < 1.0, a relative SC (compared to CC) sensitivity for ≥ CIN2 detection of 92 % (95 % CI: 88, 96) for the ratio ≥ 1.0 group and 97 % (95 % CI: 94, 100; p < 0.05) for the ratio < 1.0 group was observed. The ratio ≥ 1.0 and < 1.0 groups had relative (to CC) specificities of 98 % (95 % CI: 93, 104) and 94 % (95 % CI: 87, 101; p = 0.3383), respectively (Table 1).

Seven (7) articles (5 articles that were included in the ≥ CIN2 *meta*-analysis) (Latsuzbaia et al., 2022; Latsuzbaia et al., 2022; El-Zein et al., 2018; Rohner et al., 2020; Stanczuk et al., 2022) and 2 solely utilized for ≥ CIN3 (Polman et al., 2019; Terada et al., 2022) from the starting 17

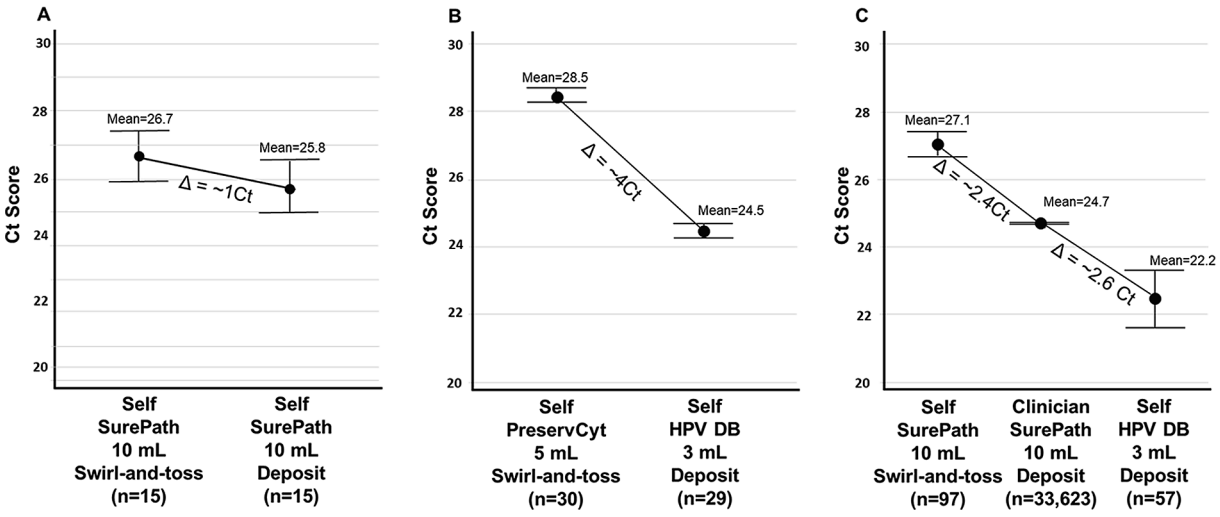

Fig. 1. (A) Impact of resuspension method (swirl-and-toss versus device deposition) on cell recovery (estimated by mean beta-globin Ct score) using the same collection device (FLOQSwab) and media volume (SurePath); (B) Impact of resuspension method and media type on cell recovery (estimated by mean beta-globin Ct score) using the same collection device (FLOQSwab) and different transport media (HPV diluent versus SurePath); (C) Comparison of the two self collection (SC) resuspension methods on cell recovery (estimated by mean beta-globin Ct score) to that of clinician-collected standard of care.

Table 1
Impact of SC-to-CC volume ratio on reported ≥ CIN2 sensitivity and specificity for HPV testing following SC.

SC: CC Vol ratio	Study	Device	Transport	SC suspension	CC Suspension	Agitation	SC: CC Vol Ratio	Number Positive	Number Negative	≥CIN2 Rel Sn (vs. CC)[a]	≥CIN2 Rel Sp (vs. CC)[a]
Volume ratio ≥ 1.0	Cho 2021 (Cho et al., 2021)[b]	Swab	Liquid	PC (20 mL)	PC (20 mL)	Swirl	1.0	129	124	0.91 [0.83, 0.99]	0.82 [0.56, 1.20]
	Cho 2021 (Cho et al., 2021)[c]	Swab	Liquid	PC (20 mL)	PC (20 mL)	Swirl	1.0	129	124	0.87 [0.78, 0.97]	0.88 [0.60, 1.27]
	El-Zein 2018 (El-Zein et al., 2018)	Swab 1	Dry	PC (20 mL)	PC (20 mL)	Swirl	1.0	152	924	0.93 [0.84, 1.02]	0.98 [0.91, 1.06]
	El-Zein 2018 (El-Zein et al., 2018)	Swab 2	Dry	PC (20 mL)	PC (20 mL)	Swirl	1.0	152	924	0.93 [0.80, 1.02]	0.93 [0.86, 1.01]
	Latsuzbaia 2022a (Latsuzbaia et al., 2022)	Brush / Swab[d]	Dry	PC (20 mL)	PC (20 mL)	Swirl	1.0	61	329	0.91 [0.80, 1.04]	1.05 [0.90, 1.22]
	Latsuzbaia 2022b (Latsuzbaia et al., 2022)	Brush / Swab[d]	Dry	PC (20 mL)	PC (20 mL)	Vortex	1.0	86	399	0.96 [0.86, 1.06]	1.08 [0.95, 1.23]
							Mean for ratio ≥ 1.0 ([95 % CI])			0.92 [0.88, 0.96]	0.98 [0.93, 1.04]
Volume ratio <1.0	Castle 2020 (Castle et al., 2020)	Brush	Liquid	PC (1 mL)	PC (20 mL)	Swirl	0.05	49	438	0.96 [0.86, 1.06]	0.90 [0.79, 1.01]
	Cocuzza 2023 (Cocuzza and Arbyn, 2021)	Swab	Dry	BHD (3 mL)	PC (20 mL)	Swirl	0.15	79	93	1.01 [0.91, 1.13]	0.95 [0.67, 1.35]
	Ertik 2021 (Ertik et al., 2021)	Brush	Dry	PC (2 mL)	PC (20 mL)	Swirl	0.1	58	7	1.00 [0.88, 1.13]	1.00 [0.30, 3.35]
	Ertik 2021 (Ertik et al., 2021)	Swab	Dry	PC (2 mL)	PC (20 mL)	Swirl	0.1	58	7	0.94 [0.82, 1.08]	1.67 [0.63, 4.42]
	Rohner 2020a (Rohner et al., 2020)	Brush	Liquid	PC (6 mL)	PC (20 mL)	Vortex	0.3	83	224	1.00 [0.93, 1.08]	0.78 [0.60, 1.01]
	Rohner 2020b (Rohner et al., 2020)	Brush	Liquid	PC (6 mL)	PC (20 mL)	Swirl	0.3	85	229	0.97 [0.88, 1.07]	0.88 [0.70, 1.10]
	Stanczuk 2022 (Stanczuk et al., 2022)	Swab	Liquid	RPM (3 mL)	PC (20 mL)	Vortex	0.15	181	4424	0.94 [0.88, 1.00]	0.98 [0.96, 0.99]
							Mean for ratio < 1.0 ([95 % CI])			0.97 [0.94, 1.00]	0.94 [0.87, 1.01]
							Combined mean ([95 % CI])			0.95 [0.92, 0.97]	0.98 [0.87, 1.01]

Abbreviations: SC, self collection; CC, clinician collection; ≥CIN2, Cervical intraepithelial neoplasia, grade 2 or worse; HPV, human papillomavirus; Vol ratio; resuspension volume ratio (SC:CC); Rel Sn, relative sensitivity; Rel Sp, relative specificity; PC, PreservCyt LBC Media; BHD, BD HPV Diluent; RPM, Roche PCR Media;
[a] Indicates performance of SC (index) for detection of ≥ CIN2 (reference) as a percent of CC (comparator) for detection of ≥ CIN2;
[b] Indicates HPV assay 1 from Cho 2022;
[c] Indicates HPV assay 2 from Cho 2022;
[d] Brush and swab specimen results were combined for performance calculations.

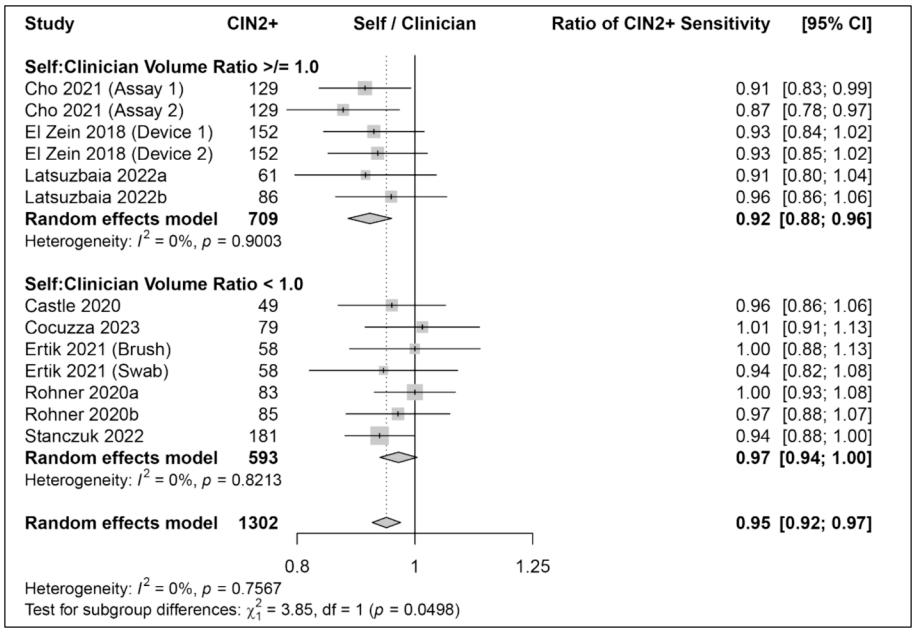

Fig. 2. Forest plot from *meta*-analysis calculating ≥ CIN2 sensitivity values for self collection-to-clinician collection volume ratio ≥ 1.0 and < 1.0.

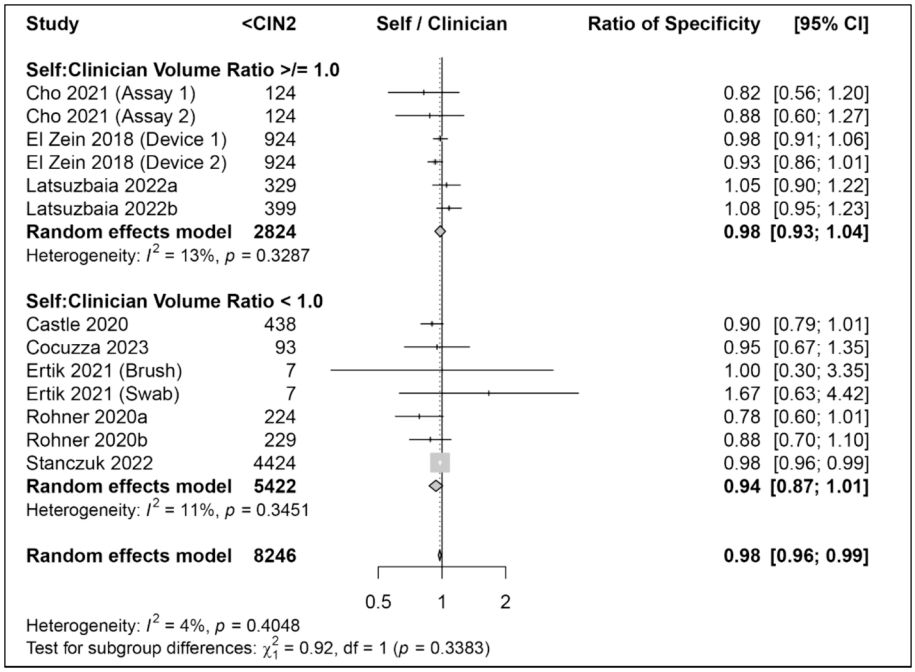

Fig. 3. Forest plot from *meta*-analysis calculating ≥ CIN2 specificity values for self collection-to-clinician collection volume ratio ≥ 1.0 and < 1.0.

articles were utilized to assess the impact of volume ratio on relative SC-to-CC ≥ CIN3 sensitivity. Too few studies were available that reported ≥ CIN3, and so a formal *meta*-analysis was not performed. However, the results (ratio ≥ 1.0 = 94 % and ratio < 1.0 = 99 %) for overall relative ≥ CIN3 sensitivity were consistent with those of using ≥ CIN2 sensitivity as an endpoint (Table S5). Five (5) of the original 17 articles reported positive percent agreement (PPA) and negative percent agreement (NPA) of SC (index) using CC as the reference. Although there were not enough articles to conduct a formal *meta*-analysis, the mean PPA and NPA for both ratio groups was calculated. Results similar to the ≥ CIN2 results were observed (ratio ≥ 1.0 group showed a PPA value of 87 %, while ratio < 1.0 group showed a PPA value of 93 %; the ratio < 1.0 group showed an NPA value of 90 %, while the ratio ≥ 1.0 group showed an NPA value of 97 %) (Table S6).

4. Discussion

Here, empirical evidence and a systematic review of recent literature revealed that resuspension volume influences assay performance; lower volumes resulted in better sensitivity than volumes closer to 10 mL or 20 mL (as with SurePath and PreservCyt, respectively) (Table 1, Table S5, and Table S6). This finding is likely explained by the natural history of high-risk HPV viruses that have a tropism for rapidly dividing cells in the transformation zone of the endocervix. One would expect to find higher viral titers in the cells of the endocervix since it is the preferred site for high-risk HPV infection. In contrast, vaginal HPV may be transitory in

nature, shed from the primary endocervical site or in transit to the transformation zone as a result of viral infection from another person. A recent study found that in positive specimens, the cervix was associated with the highest viral load (estimated using the signal-to-cutoff ratio of the Hybrid Capture 2 assay) and that the endocervical specimens had the highest ≥ CIN2 sensitivity (100 %) versus specimens from the upper vagina, lower vagina, and perineum (97.87 %, 95.74 %, and 91.49 %, respectively). (Li et al., 2023) Thus, vaginal specimens are reliable for HPV detection but vaginal collection workflows need to be developed and validated independently of endocervical specimen collection workflows, in order to optimize the proper clinical cutoffs (for both sensitivity and specificity).

Although previous *meta*-analyses have demonstrated that SC has non-inferior sensitivity to CC samples for cervical cancer screening, some studies have demonstrated diminished HPV assay performance for precancer detection following SC (Arbyn et al., 2018). However, closer examination reveals that some studies reporting reduced performance following SC also have suboptimal SC workflows. Workflow variables include the type of assay detection technologies (as DNA-based PCR is more sensitive than signal amplification and RNA-based technologies), type of resuspension media (e.g., preservative-based fixatives and preservatives providing more robust performance than phosphate-buffered saline or unpreserved urine samples), and collection device-related performance issues, which can result in variable target collection (Arbyn et al., 2018; Latsuzbaia et al., 2022; Cadman et al., 2021). To ensure consistent performance, the SC specimen has to be reliably collected, stored, and transported prior to detection with a sensitive assay method.

The empirical testing performed in this study, using different resuspension volumes and transport methods, provides a scientific rationale for some published differences between endocervical and vaginal HPV test results and are summarized in Table S7. Paired-test results using the same resuspension methods and transport media volumes result in reduced positivity and clinical sensitivity with vaginal samples. This was observed in a large, real-world implementation in the Dutch national screening program, where vaginal samples deposited in 20 mL of PreservCyt media had increased Ct scores and reduced ≥ CIN2 sensitivity compared to CC endocervical samples (Inturrisi et al., 2021). Device deposition in transport media increases positivity versus swirl-and-toss methodology; the latter results in target loss. The decreased viral load in the vagina can be offset by resuspending the collection device in a smaller volume and through the use of lytic preservative media to enhance in target acquisition. These observations explain why the preponderance of clinical data supports the non-inferiority sensitivity of vaginal SC samples compared to CC endocervical samples, while at the same time providing a logical explanation why these criteria are not always met when the SC workflow is not optimized for the HPV microbiome that exists in the vaginal canal. Swirl-and-toss methodology, during which, the collection device is discarded, results in an approximately 50 % reduction in cell transport versus depositing the device in the vial. This is expected to influence both the total target yield and the reproducibility of detection – HPV is a non-lytic virus that tends to exist in localized tissue, ultimately giving rise to discrete CIN2/3 lesions within a histology section (de Sanjose et al., 2017; Quint et al., 2012). Thus, recovering all the cells collected is likely to result in more robust performance. These hypotheses were confirmed with empirical testing of the same mock sample matrix, where direct deposit of the device in 3 mL HPV DB (lytic media) resulted in an approximately 16-fold increase in cell transport versus a swirl-and-toss method into 5 mL of PreservCyt media. This result was confirmed using SurePath media where an even larger (approximately 5 Ct) delta in recovery corresponding to about a 32-fold (2^5) difference was observed. When compared to transport from a CC 10 mL sample with brush deposited in the vial (per standard of care), the 3 mL direct deposit in lytic buffer sample demonstrates an approximately 2 Ct or 4-fold increase in cell recovery. Lytic media may be preferable to liquid-based cytology media for recovery of SC vaginal samples since cytology is not being performed on vaginal samples (they lack an endocervical component) and therefore no requirement exists to maintain cell integrity for cytology (Toliman et al., 2019). Lytic media, is the traditional choice for cell transport for downstream nucleic acid detection. If liquid-based cytology is used, care should be taken to reduce the cell transport volume to compensate for the difference in viral load in the vaginal canal. This was recently investigated by Connor et al using serial dilutions of an HPV16-positive cell line and found that larger resuspension volumes led to decreased HPV detection and concluded that devices should be resuspended in 5 mL or less of liquid media (Connor et al., 2023). Interestingly, these authors observed a device-dependent difference between vortexing and manual (swirl-and-toss) resuspension methods, with vortexing resulting in less HPV detection with the Evalyn brush but not with the FLOQSwabs, where cells were both prepared and recovered in PreservCyt media. This may be related to the chemical composition of PreservCyt fixative media, which has been reported to bind samples to the collection device after prolonged exposure and explains why depositing the collection device in PreservCyt is contraindicated for CC endocervical samples (ThinPrep® Pap Test PreservCyt® Solution, 2019; UWHealth, 2016). This is in contrast to SurePath media which uses a different fixative chemistry and therefore the device is left in the vial to maximize cell recovery (BD SurePath™ Collection [package insert]. Becton, , 2011; Bigras et al., 2003). Attention to these critical parameters when developing SC workflows will ensure robust performance and one that is equivalent in clinical performance to CC samples.

5. Conclusions and perspectives

Global cervical cancer screening programs are pivoting from cytology toward primary HPV screening, which is now recommended by WHO and a growing number of national screening guidelines (Arbyn et al., 2021; Fontham et al., 2020; WHO, 2021). CC is being supplemented with SC as means of reaching women who are under or never screened (Ejegod et al., 2022; Serrano et al., 2022). The proportion of SC versus CC samples will continue to increase as cervical cancer screening programs move from pilot to implementation phase and offer it as a more convenient option to women who attend routine screening. It is important that patient safety remains a top priority during this transition and that SC methods undergo appropriate validation as outlined in the VALHUDES protocol. The critical parameters established here will help avoid performance issues and ensure consistent results.

It is important to offset the difference in viral load between vaginal and endocervical collection sites by resuspending vaginal samples in reduced buffer volumes and depositing the collection device in the media to maximize cell recovery. Lytic media also results in an increase in cell recovery versus fixative media. The optimized workflow described here, in which the collection device is placed directly in 3 mL of lytic media, has been validated in a successful real-world implementation in the Capital Region of Denmark (Ejegod et al., 2022; Cuschieri et al., 2021; Lam et al., 2018). To date, tens of thousands of women have been screened using the Evalyn Brush deposited in 3 mL of HPV DB. The workflow has been found to be robust with a low invalid rate (0.18 %) (Cuschieri et al., 2021). The results from this study support those from previous studies and provide further impetus to continue to identify factors that can be modified in order to optimize the SC workflow in order to ensure good clinical performance during HPV testing from vaginal specimens.

Funding

This work was supported by Becton, Dickinson and Company, BD Life Sciences – Diagnostic Solutions (7 Loveton Circle, Sparks, MD 21152, USA).

Author contributions

All authors contributed to the interpretation of the data, critically revised the manuscript for important intellectual content, and approved the final version to be published. Becton, Dickinson and Company employees that are also authors played the following roles during the study and development of the paper: LV, MS, LL, and LG designed and conducted empirical studies and analyzed and interpreted the data. LV and DG facilitated study design and implementation, and revision of the manuscript; LV, DG, and VP facilitated data analysis and/or interpretation for the manuscript. All authors provided final approval of the manuscript and agree to be accountable for the accuracy and integrity of this work.

Potential conflicts of interest

LV, DG, VP, MS, LL, and LG are employees of Becton, Dickinson and Company, the sponsor of the study.

CRediT authorship contribution statement

Laurence Vaughan: Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Devin S. Gary:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization. **Millie Shah:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lyndsay Lewellen:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Laura Galbraith:** Writing – review & editing, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Valentin Parvu:** Writing – review & editing, Visualization, Validation, Software, Formal analysis.

Acknowledgements

The authors would like to thank Jeffrey Andrews, MD and Dorsey Mills (both of Becton, Dickinson and Company, BD Life Sciences – Diagnostic Solutions) for their input on the content of this manuscript and editorial assistance, respectively. JA and DM have no additional funding or additional compensation to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gore.2024.101421.

References

Arbyn, M., Smith, S.B., Temin, S., Sultana, F., Castle, P., 2018. Detecting cervical precancer and reaching underscreened women by using HPV testing on self samples: updated meta-analyses. BMJ (clinical Research Ed). 363, k4823 https://doi.org/10.1136/bmj.k4823.

Arbyn, M., Peeters, E., Benoy, I., Vanden Broeck, D., Bogers, J., De Sutter, P., et al., 2018. VALHUDES: A protocol for validation of human papillomavirus assays and collection devices for HPV testing on self-samples and urine samples. J. Clin. Virol. 107, 52–56. https://doi.org/10.1016/j.jcv.2018.08.006.

Arbyn, M., Gultekin, M., Morice, P., Nieminen, P., Cruickshank, M., Poortmans, P., et al., 2021. The European response to the WHO call to eliminate cervical cancer as a public health problem. Int. J. Cancer 148, 277–284. https://doi.org/10.1002/ijc.33189.

Arbyn, M., Castle, P.E., Schiffman, M., Wentzensen, N., Heckman-Stoddard, B., Sahasrabuddhe, V.V., 2022. Meta-analysis of agreement/concordance statistics in studies comparing self- vs clinician-collected samples for HPV testing in cervical cancer screening. Int. J. Cancer 151, 308–312. https://doi.org/10.1002/ijc.33967.

BD SurePath™ Collection [package insert]. Becton, Dickinson and Company, Burlington, NC; 2011. DOI.

Benard, V.B., Jackson, J.E., Greek, A., Senkomago, V., Huh, W.K., Thomas, C.C., et al., 2021. A population study of screening history and diagnostic outcomes of women with invasive cervical cancer. Cancer Med. 10, 4127–4137. https://doi.org/10.1002/cam4.3951.

Bigras, G., Rieder, M.A., Lambercy, J.M., Kunz, B., Chatelain, J.P., Reymond, O., et al., 2003. Keeping collecting device in liquid medium is mandatory to ensure optimized liquid-based cervical cytologic sampling. J Low Genit Tract Dis. 7, 168–174. https://doi.org/10.1097/00128360-200307000-00003.

Bottari, F., Iacobone, A.D., 2019. Profile of the BD HPV OnclarityTM assay. Expert Rev. Mol. Diagn. 19, 565–570. https://doi.org/10.1080/14737159.2019.1622415.

Bruni, L., Serrano, B., Roura, E., Alemany, L., Cowan, M., Herrero, R., et al., 2022. Cervical cancer screening programmes and age-specific coverage estimates for 202 countries and territories worldwide: a review and synthetic analysis. Lancet Glob. Health 10, e1115–e1127. https://doi.org/10.1016/s2214-109x(22)00241-8.

Cadman, L., Reuter, C., Jitlal, M., Kleeman, M., Austin, J., Hollingworth, T., et al., 2021. A randomized comparison of different vaginal self-sampling devices and urine for human papillomavirus testing-predictors 5.1. Cancer Epidemiol Biomarkers Prev. 30, 661–668. https://doi.org/10.1158/1055-9965.Epi-20-1226.

Castle, P.E., Ajeh, R., Dzudie, A., Kendowo, E., Fuhngwa, N., Simo-Wambo, A.G., et al., 2020. A comparison of screening tests for detection of high-grade cervical abnormalities in women living with HIV from Cameroon. Infectious Agents and Cancer. 15, 45. https://doi.org/10.1186/s13027-020-00311-w.

Cho, H.W., Hong, J.H., Min, K.J., Ouh, Y.T., Seong, S.J., Moon, J.H., et al., 2021. Performance and diagnostic accuracy of human papillomavirus testing on self-collected urine and vaginal samples in a referral population. Cancer Res Treat. 53, 829–836. https://doi.org/10.4143/crt.2020.1165.

Cocuzza, C., Arbyn, M. 2023. Personal communication. Sensitivity and specificity of HPV assays for high-grade cervical precancer on vaginal self-samples compared to cervical samples taken by a clinician. November 10, 2021. 2023. DOI.

Connor, L., Elasifer, H., Sargent, A., Bhatia, R., Graham, C., Cuschieri, K., 2023. Influence of resuspension volume on dry sampling devices taken for human papillomavirus testing: implications for self-sampling. Biotechniques 74, 77–84. https://doi.org/10.2144/btn-2022-0084.

Cuschieri, K., Wilson, A., Palmer, T., Stanczuk, G., Bhatia, R., Ejegod, D., et al., 2021. The challenges of defining sample adequacy in an era of HPV based cervical screening. J Clin Virol. 137, 104756 https://doi.org/10.1016/j.jcv.2021.104756.

De Pauw, H., Donders, G., Weyers, S., De Sutter, P., Doyen, J., Tjalma, W.A.A., et al., 2021. Cervical cancer screening using HPV tests on self-samples: attitudes and preferences of women participating in the VALHUDES study. Arch Public Health. 79, 155. https://doi.org/10.1186/s13690-021-00667-4.

de Sanjose, S., Brotons, M., Pavon, M.A., 2017. The natural history of human papillomavirus infection. Best Pract Res Clin Obstet Gynaecol. https://doi.org/10.1016/j.bpobgyn.2017.08.015.

Eamratsameekool, W., Phumiressunthon, K., Sukprasert, L., Pukdeesamai, P., 2023. Comparison of self- to provider-collected cervical screening with HPV DNA test at roi et province, thailand during COVID-19 pandemic. J. Med. Assoc. Thai. 106, 8–13. https://doi.org/10.35755/jmedassocthai.2023.01.13727.

Ejegod, D.M., Pedersen, H., Pedersen, B.T., Serizawa, R., Bonde, J., 2022. Operational experiences from the general implementation of HPV self-sampling to Danish screening non-attenders. Prev Med. 160, 107096 https://doi.org/10.1016/j.ypmed.2022.107096.

El-Zein, M., Bouten, S., Louvanto, K., Gilbert, L., Gotlieb, W., Hemmings, R., et al., 2018. Validation of a new HPV self-sampling device for cervical cancer screening: the cervical and self-sample in screening (CASSIS) study. Gynecol Oncol. 149, 491–497. https://doi.org/10.1016/j.ygyno.2018.04.004.

Ertik, F.C., Kampers, J., Hulse, F., Stolte, C., Bohmer, G., Hillemanns, P., et al., 2021. CoCoss-Trial: Concurrent Comparison of Self-Sampling Devices for HPV-Detection. Int J Environ Res Public Health. 18 https://doi.org/10.3390/ijerph181910388.

Esber, A., Norris, A., Jumbe, E., Kandodo, J., Nampandeni, P., Reese, P.C., et al., 2018. Feasibility, validity and acceptability of self-collected samples for human papillomavirus (HPV) testing in rural Malawi. Malawi Med J. 30, 61–66. https://doi.org/10.4314/mmj.v30i2.2.

Fontham, E.T.H., Wolf, A.M.D., Church, T.R., Etzioni, R., Flowers, C.R., Herzig, A., et al., 2020. Cervical cancer screening for individuals at average risk: 2020 guideline update from the American Cancer Society. CA Cancer J. Clin. 70, 321–346. https://doi.org/10.3322/caac.21628.

Ilardo, C., Marguerettaz, M., Breton, A., Azzoug, T., Schmitt, N., Fournier, R., et al., 2022. Performance and pre-analytical stability of self-collected samples versus clinician cervical samples for the detection of HPV16, HPV18 and a pool of 12 other HPV types on the Roche Cobas 8800 System. New Microbiol. 45, 111–114.

Inturrisi, F., Aitken, C.A., Melchers, W.J.G., van den Brule, A.J.C., Molijn, A., Hinrichs, J. W.J., et al., 2021. Clinical performance of high-risk HPV testing on self-samples versus clinician samples in routine primary HPV screening in the Netherlands: An observational study. Lancet Reg Health Eur. 11, 100235 https://doi.org/10.1016/j.lanepe.2021.100235.

Lam, J.U.H., Elfstrom, K.M., Ejegod, D.M., Pedersen, H., Rygaard, C., Rebolj, M., et al., 2018. High-grade cervical intraepithelial neoplasia in human papillomavirus self-sampling of screening non-attenders. Br. J. Cancer 118, 138–144. https://doi.org/10.1038/bjc.2017.371.

Latsuzbaia, A., Vanden Broeck, D., Van Keer, S., Weyers, S., Donders, G., Doyen, J., et al., 2022. Validation of BD onclarity HPV assay on vaginal self-samples versus cervical samples using the VALHUDES protocol. Cancer Epidemiol Biomarkers Prev. 31, 2177–2184. https://doi.org/10.1158/1055-9965.EPI-22-0757.

Latsuzbaia, A., Vanden Broeck, D., Van Keer, S., Weyers, S., Tjalma, W.A.A., Doyen, J., et al., 2022. Clinical performance of the realtime high risk HPV assay on self-collected vaginal samples within the VALHUDES framework. Microbiol Spectr. 10, e0163122 https://doi.org/10.1128/spectrum.01631-22.

Latsuzbaia, A., Broeck, D.V., Van Keer, S., Weyers, S., Donders, G., Doyen, J., et al., 2023. Comparison of the clinical accuracy of Xpert HPV assay on vaginal self-samples and

cervical clinician-taken samples within the VALHUDES framework. J Mol Diagn. https://doi.org/10.1016/j.jmoldx.2023.06.004.

Li, T., Chen, S., Li, X., Wu, Z., Zhao, Y., Cui, J., et al., 2023. The features of high-risk human papillomavirus infection in different female genital sites and impacts on HPV-based cervical cancer screening. Virol. J. 20, 116. https://doi.org/10.1186/s12985-023-02073-4.

Martinelli, M., Giubbi, C., Sechi, I., Bottari, F., Iacobone, A.D., Musumeci, R., et al., 2022. Evaluation of BD onclarity HPV assay on self-collected vaginal and first-void urine samples as compared to clinician-collected cervical samples: a pilot study. Diagnostics (Basel) 12. https://doi.org/10.3390/diagnostics12123075.

Martinelli, M., Latsuzbaia, A., Bonde, J., Pedersen, H., Iacobone Anna, D., Bottari, F., et al., 2024. Performance of BD Onclarity HPV assay on FLOQSwabs vaginal self-samples. Microbiol Spectr. e02872–e10923. https://doi.org/10.1128/spectrum.02872-23.

Meijer, C.J., Berkhof, J., Castle, P.E., Hesselink, A.T., Franco, E.L., Ronco, G., et al., 2009. Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older. Int. J. Cancer 124, 516–520. https://doi.org/10.1002/ijc.24010.

Onuma, T., Kurokawa, T., Shinagawa, A., Chino, Y., Yoshida, Y., 2020. Evaluation of the concordance in HPV type between self- and physician-collected samples using a brush-based device and a PCR-based HPV DNA test in Japanese referred patients with abnormal cytology or HPV infection. Int J Clin Oncol. 25, 1854–1860. https://doi.org/10.1007/s10147-020-01727-5.

Polman, N.J., Ebisch, R.M.F., Heideman, D.A.M., Melchers, W.J.G., Bekkers, R.L.M., Molijn, A.C., et al., 2019. Performance of human papillomavirus testing on self-collected versus clinician-collected samples for the detection of cervical intraepithelial neoplasia of grade 2 or worse: a randomised, paired screen-positive, non-inferiority trial. Lancet Oncol. 20, 229–238. https://doi.org/10.1016/S1470-2045(18)30763-0.

Quint, W., Jenkins, D., Molijn, A., Struijk, L., van de Sandt, M., Doorbar, J., et al., 2012. One virus, one lesion–individual components of CIN lesions contain a specific HPV type. J Pathol. 227, 62–71. https://doi.org/10.1002/path.3970.

Rohner, E., Rahangdale, L., Sanusi, B., Knittel, A.K., Vaughan, L., Chesko, K., et al., 2020. Test accuracy of human papillomavirus in urine for detection of cervical intraepithelial neoplasia. J Clin Microbiol. 58 https://doi.org/10.1128/JCM.01443-19.

Rohner, E., Edelman, C., Sanusi, B., Schmitt, J.W., Baker, A., Chesko, K., et al., 2020. Extended HPV genotyping to compare HPV type distribution in self- and provider-collected samples for cervical cancer screening. Cancer Epidemiol Biomarkers Prev. 29, 2651–2661. https://doi.org/10.1158/1055-9965.EPI-20-0674.

Serrano, B., Ibáñez, R., Robles, C., Peremiquel-Trillas, P., de Sanjosé, S., Bruni, L., 2022. Worldwide use of HPV self-sampling for cervical cancer screening. Prev. Med. 154. https://doi.org/10.1016/j.ypmed.2021.106900.

Serrano, B., Ibáñez, R., Robles, C., Peremiquel-Trillas, P., de Sanjosé, S., Bruni, L., 2022. Worldwide use of HPV self-sampling for cervical cancer screening. Prev Med. 154, 106900 https://doi.org/10.1016/j.ypmed.2021.106900.

Stanczuk, G.A., Currie, H., Forson, W., Baxter, G., Lawrence, J., Wilson, A., et al., 2022. Self-sampling as the principal modality for population based cervical screening: Five-year follow-up of the PaVDaG study. Int. J. Cancer 150, 1350–1356. https://doi.org/10.1002/ijc.33888.

Stoler, M.H., Wright Jr., T.C., Parvu, V., Vaughan, L., Yanson, K., Eckert, K., et al., 2018. The onclarity human papillomavirus trial: design, methods, and baseline results. Gynecol Oncol. 149, 498–505. https://doi.org/10.1016/j.ygyno.2018.04.007.

Terada, N., Matsuura, M., Kurokawa, S., Nishimura, Y., Tamate, M., Isoyama, K., et al., 2022. Human papillomavirus testing and cytology using physician-collected uterine cervical samples vs. self-collected vaginal samples and urine samples. Int J Clin Oncol. 27, 1742–1749. https://doi.org/10.1007/s10147-022-02238-1.

ThinPrep® Pap Test PreservCyt® Solution [package insert]. Hologic, Inc. Marlborough, MA; 2019. DOI.

Toliman, P.J., Phillips, S., de Jong, S., O'Neill, T., Tan, G., Brotherton, J.M.L., et al., 2019. Evaluation of p16/Ki-67 dual stain cytology performed on self-collected vaginal and clinician-collected cervical specimens for the detection of cervical pre-cancer. Clin Microbiol Infect. https://doi.org/10.1016/j.cmi.2019.10.020.

Tranberg, M., Jensen, J.S., Bech, B.H., Blaakær, J., Svanholm, H., Andersen, B., 2018. Good concordance of HPV detection between cervico-vaginal self-samples and general practitioner-collected samples using the Cobas 4800 HPV DNA test. BMC Infect. Dis. 18, 348. https://doi.org/10.1186/s12879-018-3254-y.

UWHealth. PAP AND HPV TESTING FREQUENTLY ASKED QUESTIONS https://bynderuwhealthorg/m/1f845e4bbdcea068/original/PAP-and-HPV-Testing-FAQpdf. 2016. DOI.

Van Keer, S., Latsuzbaia, A., Vanden Broeck, D., De Sutter, P., Donders, G., Doyen, J., et al., 2022. Analytical and clinical performance of extended HPV genotyping with BD Onclarity HPV Assay in home-collected first-void urine: A diagnostic test accuracy study. J Clin Virol. 155, 105271 https://doi.org/10.1016/j.jcv.2022.105271.

WHO: New recommendations for screening and treatment to prevent cervical cancer. https://www.who.int/news/item/06-07-2021-new-recommendations-for-screening-and-treatment-to-prevent-cervical-cancer, 2021. DOI.

Young, S., Vaughan, L., Yanson, K., Eckert, K., Li, A., Harris, J., et al., 2020. Analytical and clinical sample performance characteristics of the onclarity assay for the detection of human papillomavirus. J Clin Microbiol. 59 https://doi.org/10.1128/jcm.02048-20.