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Efficacy and acceptability of a self-collected medical grade tampon as a novel vaginal sample collection tool for the detection of HPV and STIs

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Abstract

Objective Cervical cancer remains a significant health concern, particularly in low-income and middle-income countries (LMICs). This study aims to compare the efficacy and suitability of a self-collected tampon for the detection of human papillomavirus (HPV) and sexually transmitted infections (STIs) using qualitative TMA-based assays (**T**ranscription **M**ediated **A**mplification; APTIMA® HPV, APTIMA® Combo 2 (CT/NG; AC2 from now on) and APTIMA®Bacterial Vaginosis (BV from now on). Additionally, we assess the acceptability of tampons as a self-collection tool.

Methods A cohort of 75 female participants aged 18–54 years was recruited through female-focused social networks. Participants provided informed consent and underwent both Health Care Workers (HCW-collected) and self-collected sample collection using the Daye Diagnostic Tampon. Samples were stored in ThinPrep Vials (TP Vial) or Aptima® Multitest Swab Collection Kit (APTIMA®) solutions. HPV and STI testing were performed using TMA-based assay on the fully automated Panther® Platform. Acceptability was assessed through a questionnaire with Likert-scale responses.

Results The study involved 60 participants who completed the study (80% of recruited participants). The self-collected tampons showed sensitivity and specificity of 66.67% and 90.74% (when rinsed in TP Vial) and 83.33% and 85.42% (when rinsed in APTIMA®) for HPV detection, respectively. For bacterial vaginosis (BV) detection, the tampons exhibited sensitivity and specificity of 100.0% and 96.43% (TP Vial) and 88.89% and 98.04% (APTIMA), respectively. For detection of chlamydia and gonorrhoea (AC2), the sensitivity and specificity were 100.00% and 100.0% (TP Vial) and 100.00% and 98.31% (APTIMA), respectively. Participants expressed a preference for tampon self-collection over HCW-collected swabs (90%).

Conclusion Self-collected tampons demonstrated promising diagnostic accuracy to HCW-collected swabs for HPV and STI detection. The tampon self-collection method was well-accepted and preferred by participants, suggesting its potential as an alternative screening tool, particularly in low-resource settings. Further research with larger and more

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diverse populations is recommended to validate these findings and inform tampon-based self-collection programs for cervical cancer screening. Randomised controlled trials and comparisons with gold standard methods would enhance validation.

Keywords Cervical cancer, HPV, STI, Self-collection, Tampon, TMA, Acceptability, Diagnostic accuracy

Introduction

Cervical cancer is the fourth most common cancer among women globally and the leading cause of cancer-related deaths in low-income and middle-income countries (LMICs) [36]. In 2020, an estimated 604,127 new cases and 341,831 deaths were attributed to cervical cancer [30], World Health Organisation, 2023). Persistent high-risk Human Papillomavirus (hrHPV) infection is the primary causative factor for cervical cancer and its precursor lesions [14, 15, 36].

Bacterial vaginosis (BV) is the most prevalent cause of abnormal vaginal discharge among women of reproductive age [17, 20]. BV is characterized by the depletion of Lactobacillus-dominant vaginal microflora and overgrowth of anaerobic bacteria [2, 17, 25]. BV has been linked to various sexually transmitted infections (STIs) and an increased susceptibility to HIV acquisition [3, 23]. Evidence suggests that BV may elevate the risk of acquiring HPV [26] due to alterations in the vaginal ecosystem [17].

Screening for HPV and cervical cancer has effectively decreased both the occurrence and death rates associated with cervical cancer, particularly in affluent settings with well-organized, widely covered, and high-quality population-based screening programs [10, 36, 37]. Effective screening initiatives require the availability and accessibility of cervical cancer screening tests, such as cervical cytology (Pap smear), visual inspection with acetic acid (VIA), or HPV testing, [24, 36]. In LMICs, Pap testing has faced challenges due to inadequate organization, limited medical coverage, and a lack of quality assurance [18, 31]. As a result, HPV testing has been endorsed as the primary method for cervical cancer screening in numerous countries [37].

The prevailing recommendations for cervical cancer screening in the majority of countries advise the utilisation of cytology and HPV tests conducted on samples collected by healthcare professionals [13, 27]. Nevertheless, these examinations pose inherent challenges, encompassing emotional hurdles like embarrassment and discomfort, along with practical issues such as time constraints and a laborious collection process [16]. In contrast, HPV testing, demonstrating equivalent validity in both self-collected and physician-collected samples, offers a solution to surmount the aforementioned obstacles [15, 34]. Self-sampling involves individuals obtaining a testing kit, self-collecting samples, and then forwarding these specimens to a designated laboratory. Following this, the laboratory analyses the self-collected samples and communicated the test results back to the individual.

Various self-sampling instruments, including brushes, swabs, and lavages, are available for testing purposes [21, 28, 31]. This approach to sample collection proves viable in both clinical settings and beyond the traditional healthcare system, with initiation possible by either healthcare providers or patients themselves [21, 28, 36]. Numerous studies have indicated that self-collection offers benefits such as reduced costs, non-invasiveness, and widespread acceptance [7].

The combined validation of molecular assays for HPV detection and self-collection devices is crucial for ensuring the reliability and effectiveness of screening programs (Arbyn, et.al., 2022). Additionally, the preanalytical phase, including sample collection, storage, and transport, significantly impacts the quality of results. As reported by Arbyn et al. [8], proper handling of specimens is essential for maintaining the integrity of nucleic acids and ensuring accurate detection of HPV and other pathogens.

Recent studies have explored the potential of menstrual tampons as a means of biospecimen collection. Adamson et al. [1] compared self-collected tampon samples to traditional endocervical swabs for high-risk human papillomavirus (hrHPV) mRNA testing, finding no significant difference in positive test results, indicating the potential viability of tampons for hrHPV testing1. However, tampon samples exhibited lower sensitivity and specificity, suggesting the need for further refinement in their application1. Tiiti et al. [32] identified a similar positivity rate for hrHPV DNA detection tool. Bakkum-Gamez et al. [11] revealed that both endometrial brushing and an intravaginal tampon yielded sufficient DNA quantities and quality for endometrial cancer detection. Tampons are familiar to many women and could potentially encourage women, previously embarrassed or scared, to explore more private, clinically validated health solutions.

Daye's Tampon Screen is an at-home kit for user collection of a vaginal sample using a medical-grade tampon, Daye's Diagnostic tampon, and storage in a specimen container for transport. The device is intended for use in conjunction with vaginal microbiome and HPV screening.

To contribute further to the body of knowledge regarding the utilisation of tampons as biospecimens for HPV testing, we conducted a comparative analysis of HPV and STI status between tampon samples and the established gold standard, which involves HCW-collected cervical swabs for HPV detection and HCW-collected low vaginal swab for STI detection. Our study aimed to evaluate both the effectiveness and acceptability of self-collected medical-grade tampons as an innovative vaginal sample collection method for the detection of HPV and STIs.

Materials and methods

Study population

This preliminary study involved a cohort of 75 female participants aged 18–54 years through questionnaires distributed in female-focused social networks. Upon enrollment, participants underwent a telephone screening process based on the inclusion and exclusion criteria outlined in Table 1. Eligible participants were provided with informed consent forms, which they reviewed and signed. The study duration spanned 3 months. Out of the 75 participants initially screened, 60 successfully completed the study.

Patients were provided with a testing kit containing comprehensive instructions for sample collection using the Daye Diagnostic Tampon. The kit also included information regarding the scheduled visit to the hospital for a clinician taken sample. During the clinic visit, a study coordinator collected both a cervical and a low vaginal swab sample from each participant, following the standard procedures for cervical and low vaginal sampling. Following the sample collection, participants were asked to complete a brief questionnaire to gather their feedback and preferences regarding the sample collection method.

Sample size calculation

Given our recruitment of patients with a previous HPV positive result, a prevalence estimate of 10% was used. Using parameters of base sensitivity 0.5, alternative sensitivity 0.95, alpha 0.05, and power 0.8, the calculated total sample size was 67 participants. Our study enrolled 60 participants, closely approximating this target. This sample size allows detection of a large difference in sensitivity (0.45) between the tampon-based and traditional collection methods, with 80% power and 5% type I error rate. While appropriate for an initial investigation of this novel collection method, we acknowledge that this sample size may limit detection of smaller differences in sensitivity. The findings should be considered preliminary, with larger studies recommended to confirm and expand upon our results.

Study design

For the clinical stage, the study employed two arms to compare different specimen collection methods (Fig. 1). In the Reporting Arm, healthcare workers collected a cervical and a low vaginal specimen from the participants. In the Experimental Arm, participants used self-collected tampons to obtain their samples. The collected samples from both arms were divided into two types of collection containers: the ThinPrep Vial (TP Vial) utilising methanol-based PreservCyt™ solution, or the Aptima Multitest Swab Collection Kit (APTIMA®) containing 2.9 ml Specimen Transport Medium (STM), Lauryl Sulfate Lithium Salt Buffered Solution. Following collection, the samples were stored at room temperature for 4–6 weeks before RNA isolation. A total of 120 samples were collected for each arm.

To assess the acceptability of the tampons, participants were provided with a questionnaire consisting of open and closed questions, as well as a 5-point Likert-style scale. This allowed the principal investigator to gather feedback on participants' experience and preference regarding the tampon as a specimen collection method.

Sample collection

Sample collection commenced between the dates 01/12/2022- 16/12/2022. Each participant was instructed to insert the tampon in their vagina, leave it in place for at least 20 min, then remove it, place it in the sterile container and return it to the nurse during the screening visit at the hospital. At each study visit in which samples were collected, the cervical and low vaginal specimens were collected by inserting the Aptima® Multitest Swab into

Table 1 Inclusion and Exclusion criteria for the clinical trial

Inclusion Criteria	Exclusion Criteria
• Females aged between 18–54; • Be in self-reported good general health and considered healthy by the Investigator; • Able to provide written informed consent, including signing and dating the informed consent form (ICF); • Is able to speak, read and write in English and/or Bulgarian	• Have difficulty using tampons; • Have had toxic shock syndrome; • Currently have an urinary tract infection; • Are currently using current antibiotics, or used antibiotics within 4 weeks prior to enrolment; • Are currently using current antifungals, or used antifungals within 4 weeks prior to enrolment;

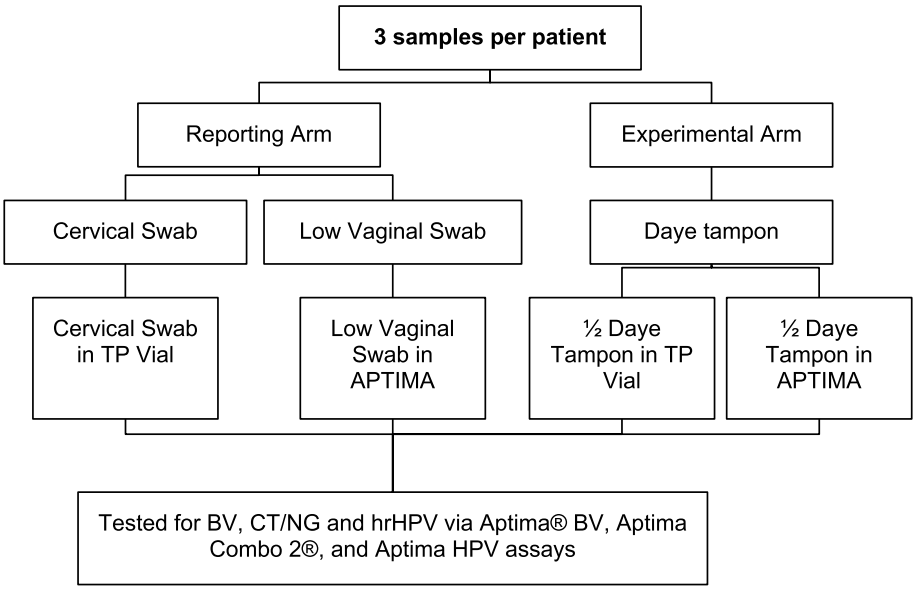

Fig. 1 Workflow for the clinical step of the study

the cervical opening (the lower part of the uterus) and low vaginal canal, respectively, and rotating five times. Both the swabs and the tampons are then divided and placed in either of the two collection solutions for storage, as shown in Fig. 1.

The tampons used for self-sampling are 100% organic cotton diagnostic tampons (Daye). The pledget, made entirely of organic cotton, is fashioned with a 'W' wadding design and protective sleeve/overwrap. A withdrawal cord, made from mercerized organic cotton, is attached to the pledget (Fig. 2). The tampon is contained inside an applicator chamber and is ejected into the vaginal canal by the user with the push of the applicator tail to ensure accurate positioning. The applicator has the same design as other products on the market in the European Union and is made from bio-based low-density polyethylene. The Daye Diagnostic Tampons are medical grade, have a protective sleeve, and are sanitised with gamma rays, unlike the traditional menstrual tampons.

Reporting arm

For the Reporting Arm, all cervical swab specimens were stored in a TP Vial containing 20 ml of PreservCyt Solution as per the Manufacturer's Instructions. The specimens were stored at temperatures ranging from 2 °C to 30 °C until testing. Upon arrival at the laboratory, they were processed according to the manufacturer's instructions. Specifically, 1 ml of the liquid was extracted and transferred to an Aptima Multitest Specimen Collection Tube for BV and AC2 testing, and another 1 ml was transferred to an Aptima Specimen Transfer Tube for HPV testing. These tubes were then loaded onto the Panther® System to perform parallel testing for AC2, HPV, and BV.

All lower vaginal swab specimens were placed directly into an Aptima Multitest Specimen Collection Tube after sample collection. These tubes contained 2.9 ml of STM lysis buffer, as per the Manufacturer's Instructions. The specimens were then stored accordingly. The Aptima Multitest Collection Tubes, equipped with pierceable cups, were directly loaded onto the Panther® System to perform simultaneous testing for AC2 and BV using a single tube that provided sufficient volume to detect all three targets in parallel (CT, NG, and BV).

Experimental arm

For the Experimental Arm, each tampon was processed to remove the outer sleeve and split it into two equal parts to ensure consistent sample distribution. Two parallel cuts were made 0.5 cm on either side of the tampon's midline, extending through the entire length of the tampon. These cuts were deep enough to separate the outer sleeve from the absorbent core. The absorbent core was then discarded. This process resulted in two equal pieces of the tampon sleeve, which were used for subsequent analysis. This method ensured that the sample was taken from the outer surface of the tampon, which had been in direct contact with the vaginal wall, while discarding the inner core that may have contained less representative material.

Half of the tampon's protective sleeve was stored in the TP Vial, containing 20 ml of PreservCyt Solution,

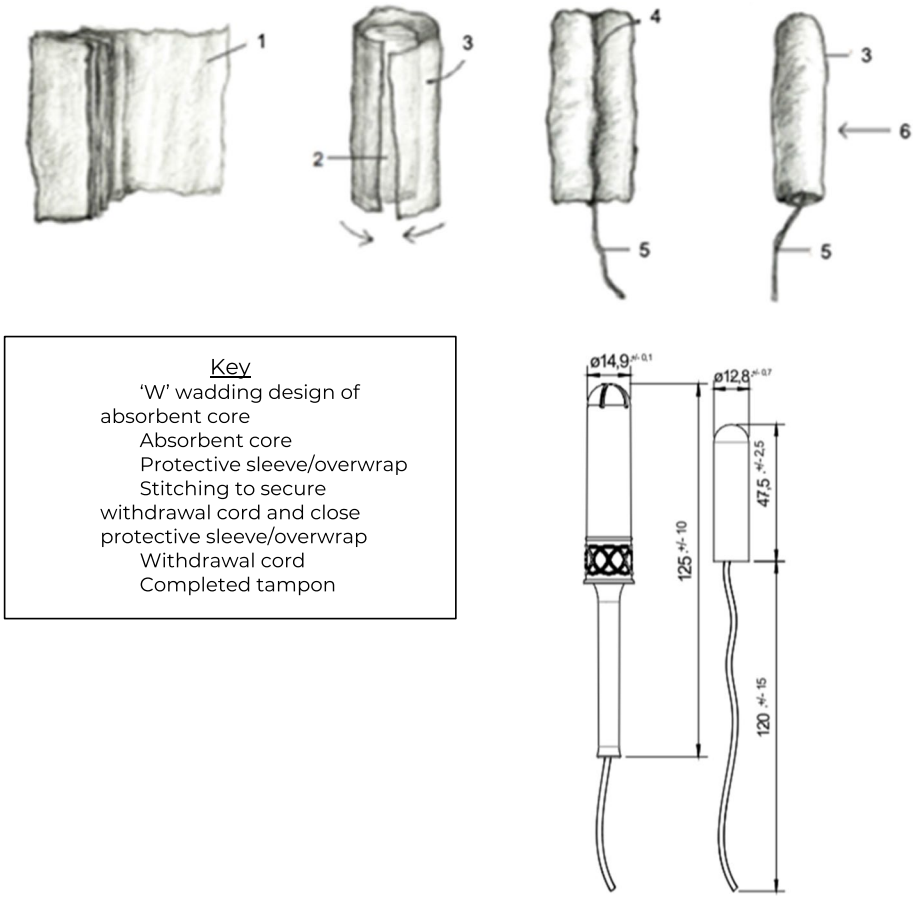

Fig. 2 Structure of the tampon and dimensions of the final device, enclosed in the applicator of the tampon as well as its raw materials

and stored according to the Manufacturer's Instructions. Similar to the Reporting Arm, 1 ml of the PreservCyt Solution liquid from the vaginal swab specimens was transferred to the Aptima Multitest Specimen Collection Tube (for AC2 and BV), and 1 ml was transferred to the Aptima Specimen Transfer Tube (for HPV), containing 2.9 ml of STM lysis buffer. The storage and testing procedures were the same as in the Reporting Arm, with AC2 (CT/NG), HPV, and BV testing performed in parallel.

The remaining half of the tampon's protective sleeve was stored in the Aptima Multitest Specimen Collection Tube, containing 2.9 ml of STM lysis buffer, and stored according to the Manufacturer's Instructions. The Aptima Multitest Collection Tubes, equipped with pierceable cups, were directly loaded onto the Panther® System, and AC2 (CT/NG), HPV, and BV testing were performed out of a single tube, containing enough volume to detect the 4 targets in parallel (CT, NG, HPV, and BV).

Testing assays

The Aptima® HPV assay detects E6/E7 mRNA of 14 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68).

For STI testing, the Aptima® BV assay, for qualitative detection of ribosomal RNAfrom bacteria associated with bacterial vaginosis (BV), including *Lactobacillus* spp. (*L. gasseri*, *L.crispatus*, and *L. jensenii*), *Gardnerella vaginalis*, and *Atopobium vaginae* and the Aptima Combo 2® assay, for the in vitro qualitative detection and differentiation of ribosomal RNA (rRNA) from *Chlamydia trachomatis* (CT) and/or *Neisseria gonorrhoeae* (GC), have been used.

Testing procedures adhered to the protocol outlined by Adamson et al. [1]. Each testing assay incorporated an internal control that monitored target capture, amplification, and detected assay steps. This control, which targets a specific human gene sequence, ensures the presence of adequate cellular material and the absence of inhibitors that could affect the accuracy of results. The internal

control was evaluated independently from the signal through the use of separate probes and light emission measurements. Test results were either positive, negative, or invalid. Invalid results were generated if the internal control surpassed a signal cutoff threshold, as specified by the manufacturer. Amplification inhibitors contaminating a specimen or the presence of a precipitate might trigger an invalid result. All invalid results were repeated; results that were invalid on two separate testing runs were reported as invalid.

Analysis

We compared the overall prevalence of hrHPV mRNA between the two collection methods (Table 2). The HCW-collected cervical swab and low vaginal swab test results were used as the reference standard for HPV and BV & AC2, respectively, to estimate the sensitivity and specificity of the tampon-collection method, with corresponding 95% confidence intervals. For each diagnostic target, the combination of the standard of care test (pathogen/condition present vs. absent) and tampon evaluation (+vs. -) provides the following contingency (Table 2).

Table 2 Shows the diagnostic performance of the tampon is evaluated according to the following parameters

		Tampon result	
		-	+
HCW-collected Swab result	**Absent**	A (True negative)	B (False positive)
	Present	C (False negative)	D (True positive)

• **Sensitivity**, the probability of correctly evaluating a case when the pathogen/condition is present: D/(C + D)

• **Specificity**, the probability of correctly evaluating a case when the pathogen/condition is absent: A/(A + B)

• **Accuracy,** the proportion of correct assessments (both true positives and true negatives) among all evaluated cases: (A + D)/(A + B + C + D)

Additionally, the prevalence of each pathogen/condition in the sample is reported (B + D)/(A + B + C + D).

We calculated 95% confidence intervals around each estimate to assess the degree of uncertainty. Acceptability of the Pap test and the tampon self-collection were assessed using a Likert scale. All statistical analyses were performed in R, version 4.2.2.

Ethics

All study procedures were explained to participants and written informed consent was obtained. All study protocols and documents were reviewed and approved by the institutional review boards at the NM Genomix, Sofia, Grad Sofia, Bulgaria.

Results

Out of the 75 subjects invited to participate there were 60 evaluable. Attrition was attributed to travel arrangements and inability to provide a sample due to menstruation. All 60 women had paired HCW-collected and self-collected specimens obtained for laboratory testing. None of the samples among the HCW-collected specimens and among the tampon specimens were excluded due to laboratory processing errors or invalid results.

HPV results

Of the 60 HCW-collected specimens that were collected and stored in the TP vial, a total of 6 samples were positive for HPV using TP Vials. Of those, 4 were positive through Tampon. In total, 54 samples were negative for HPV, 49 of which were also classified as negative through the tampon (Fig. 3). For the assessment using Aptima Tubes, 10 out of 12 samples were correctly classified as positive through the Tampon, and 41 out of 48 correctly assessed as negative (Fig. 3).

For the TP vial sample collection methods, the sensitivity and specificity of the self-collected tampons were 66.67% (95% CI: 22.3% -95.7%) and 90.74% (95%

Fig. 3 Negative and Positive results for HPV in tampons collected in TP Vial and APTIMA Tube vs TP Vial and APTIMA Tube alone

CI: 79.7%-96.9%), respectively using HCW-collected specimens as the reference. For the Aptima Multitest Swab Collection Kit, the sensitivity and specificity of the self-collected tampons were 83.33% (95% CI: 51.6%-97.9%) and 85.42% (95% CI: 72.2%- 93.9%), respectively (Table 3).

BV results

Of the 60 HCW-collected specimens that were collected and stored in the TP vial, a total of 4 samples were tested positive for BV using TP Vials. Of those, all 4 were also evaluated as positive through the Tampon. In total, 56 samples were negative for BV, 54 of which were also classified as negative through the tampon (Fig. 4). For the assessment using Aptima Tubes, 8 out of 9 samples were correctly classified as positive through the Tampon, and 50 out of 51 correctly assessed as negative (Fig. 4).

For the TP vial sample collection methods, the sensitivity and specificity of the self-collected tampons were 100.0%% (95% CI: 39.8%- 100.0%) and 96.43% (95% CI: 87.7%- 99.6%), respectively using HCW-collected specimens as the reference. For the Aptima Multitest Swab Collection Kit, the sensitivity and specificity of the self-collected tampons were 88.89% (95% CI: 51.8%-99.7%) and 98.04% (95% CI: 89.6%- 100.0%), respectively (Table 4).

Table 3 Diagnostic Accuracy of Daye Tampon used in combination with Hologic's TP Vial & Aptima Multitest for HPV detection

Parameter	Tampon in TP Vial	95% CI	Tampon in Aptima Tube	95% CI
Sensitivity	66.67%	22.3%-95.7%	83.33%	51.6%-97.9%
Specificity	90.74%	79.7%-96.9%	85.42%	72.2%-93.9%
Accuracy	88.33%	77.4%-95.2%	85.00%	73.4%-92.9%

Table 4 Diagnostic Accuracy of Daye Tampon used in combination with Hologic's TP Vial & Aptima Multitest for BV detection

Parameter	Tampon in TP Vial	95% CI	Tampon in Aptima Tube	95% CI
Sensitivity	100.00%	39.8%-100.0%	88.89%	51.8%-99.7%
Specificity	96.43%	87.7%-99.6%	98.04%	89.6%-100.0%
Accuracy	96.67%	88.5%-99.6%	96.67%	88.5%-99.6%

AC2 results

Of the 60 HCW-collected specimens that were collected and stored in the TP vial, only 1 sample was tested positive for AC2 using TP Vials. This one sample was also evaluated as positive through the tampon. In total, 59 samples were negative for AC2, all of which were also classified as negative through the tampon (Fig. 5). For the assessment using Aptima Tubes, the one positive sample was correctly classified as positive through the Tampon. In total, 59 samples were negative for AC2, 58 of which were also assessed as negative through the tampon (Fig. 5).

For the TP vial sample collection methods, the sensitivity and specificity of the self-collected tampons were 100.00% (95% CI:2.5%- 100.0%) and 100.0% (95% CI: 93.9%- 100.0%), respectively using HCW-collected specimens as the reference. While for the Aptima Multitest Swab Collection Kit, the sensitivity and specificity of the self-collected tampons were 100.00% (95% CI:2.5%-100.0%) and 98.31% (95% CI: 90.9%- 100.0%), respectively (Table 5).

Acceptability of collection methods

There were 35 women (87.5%) who were already familiar with using tampons and 5 women (12.5%) reported that the instructions prepared them well for the tampon

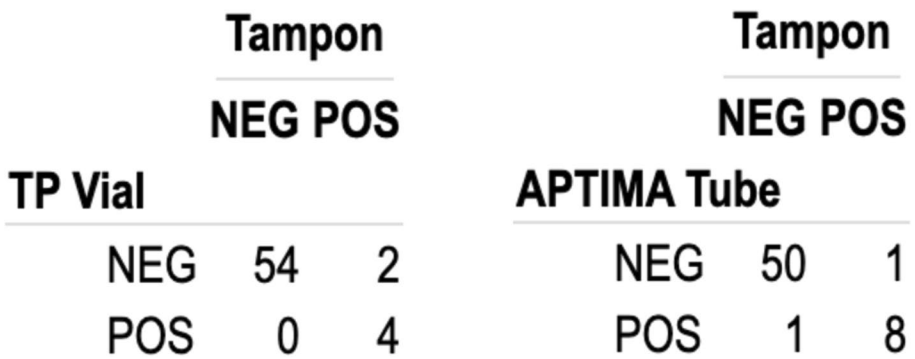

Fig. 4 Negative and Positive results for Bacterial Vaginosis(BV) in tampons collected in TP Vial and APTIMA Tube vs TP Vial and APTIMA Tube alone

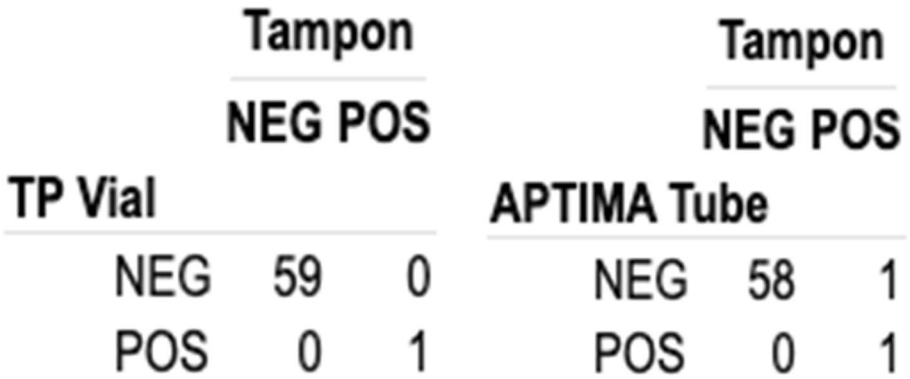

Fig. 5 Negative and Positive results for AC2 in tampons collected in TP Vial and APTIMA Tube vs TP Vial and APTIMA Tube alone

Table 5 Diagnostic Accuracy of Daye Tampon used in combination with Hologic's TP Vial & Aptima Multitest for AC2 detection

Parameter	TP Vial	95% CI	Aptima Tube	95% CI
Sensitivity	100.00%	2.5%-100.0%	100.00%	2.5%-100.0%
Specificity	100.00%	93.9%-100.0%	98.31%	90.9%-100.0%
Accuracy	100.00%	94.0%-100.0%	98.33%	91.1%-100.0%

collection. When asked to choose one collection method, the majority of participants (90%) expressed a preference for the tampon self-collection method over the HCW-collected swab, including both cervical and lower vaginal swabs (see Fig. 6).

Participants' feelings towards using menstrual tampons and undergoing gynaecological exams for sampling were assessed (see Fig. 7). Responses were collected on a

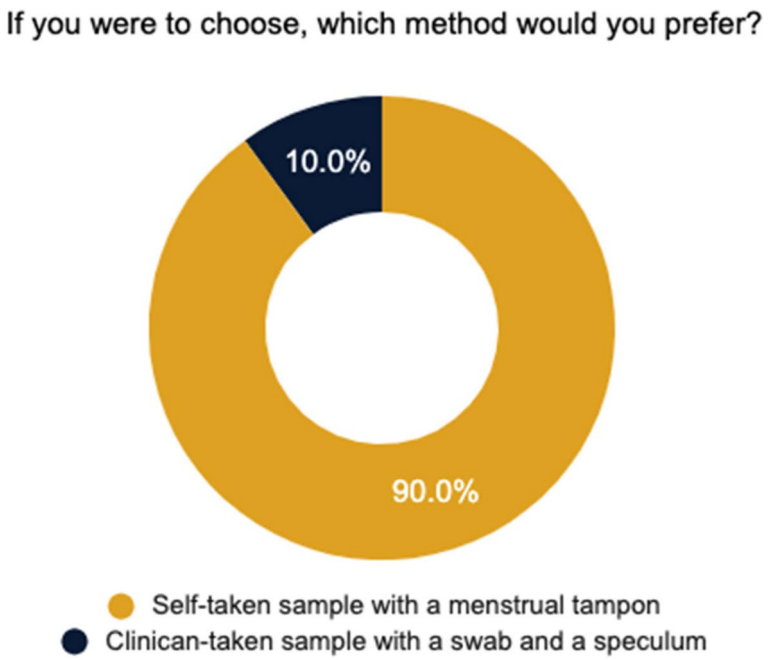

Fig. 6 Patient Preference for Sample Collection Method. The pie chart shows that 90% of survey respondents preferred self-collected tampon samples over clinician-collected swab samples with a speculum, indicating a strong patient preference for the tampon-based method

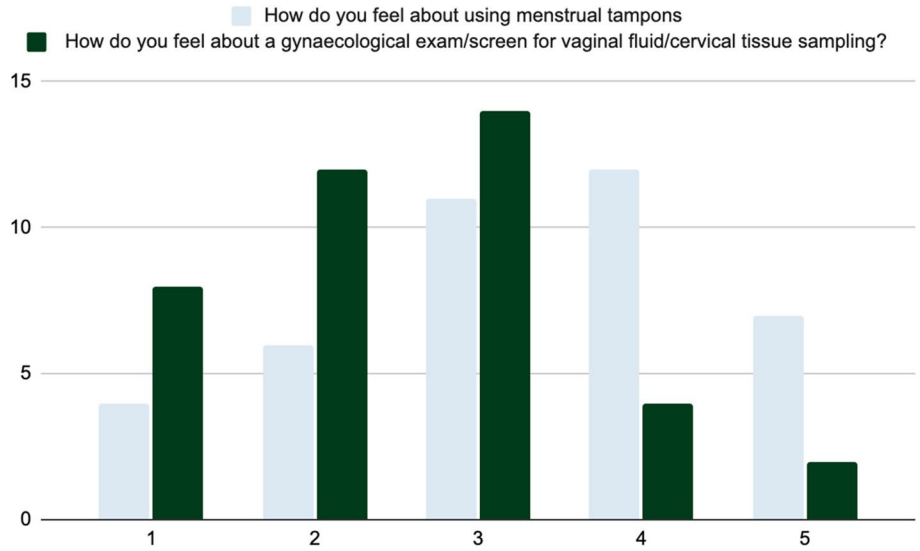

Fig. 7 Distribution of Participant Responses Regarding Sample Collection Methods. This graph shows the distribution of participant responses on a 5-point Likert scale, comparing feelings towards using menstrual tampons and undergoing gynaecological exams for vaginal fluid/cervical tissue sampling. The x-axis represents Likert scale ratings from 1 (very negative) to 5 (very positive), while the y-axis shows the number of respondents for each rating. The graph demonstrates a generally more positive attitude towards using menstrual tampons compared to gynaecological exams

5-point Likert scale, where 1 indicated very negative feelings and 5 indicated very positive feelings.

Regarding the use of menstrual tampons, the median response was 3 (neutral), with a mode of 4 (positive). A majority of respondents (51.3%) reported positive or very positive feelings (4 or 5 on the Likert scale) towards using menstrual tampons, while 25.7% reported negative or very negative feelings (1 or 2 on the Likert scale).

For gynaecological exams/screens for sampling, the median and mode were both 3 (neutral). Only 25% of respondents reported positive or very positive feelings towards these exams, while 37.5% reported negative or very negative feelings. The largest group (37.5%) expressed neutral feelings.

These results suggest that participants generally felt more positive about using menstrual tampons compared to undergoing gynaecological exams for sampling. This preference supports the potential acceptability of tampon-based self-sampling methods for vaginal microbiome analysis.

Discussion

Cervical cancer remains a significant global health concern, particularly in LMICs, where it is a leading cause of cancer-related deaths in women. The oncogenic HPV infection is central to this challenge, primarily triggering the onset of this malignancy1. Therefore, developing accurate and accessible HPV detection methods is crucial for effective screening programmes.

This study aimed to comparatively evaluate the efficiency and suitability of a self-administered, medical-grade tampon (Daye's Diagnostic Tampon) against a cervical swab obtained by a HCW for detecting HPV and STIs. This comparison assesses the diagnostic tampon's performance and its potential roles in advancing early detection and prevention strategies for cervical cancer and STIs.

Tampons offer potential benefits over urine or vaginal swabs for sample collection1. They are familiar to many women and may provide more comprehensive sampling of the vaginal environment. Previous studies have shown that tampon-based sampling is well-accepted by women [33].

Our study demonstrated that self-collected tampon specimens exhibited comparable diagnostic accuracy to HCW-collected swab specimens in detecting HPV, BV, CT, and NG. However, the sensitivity and specificity of self-collected tampons varied depending on the collection method and storage solution used.

The collection method and storage solution used for the tampons influenced the overall detection rate. The APTIMA Tube showed a two-fold increase in HPV detection compared to the TP Vial (APTIMA tube: 20%, TP Vial: 10%). These results can be attributed to the different sample dilution factors in the distinct workflows:

- APTIMA Tube: 2.9 ml of STM, with 0.4 ml being tested

- TP Vial: ~ sevenfold higher primary dilution (20 ml vs. 3 ml) and a secondary 4× dilution (1 ml of TP Vial in 2.9 ml of STM), with 0.4 ml tested

The dilution effect on the detection rate was confirmed using unused tampons spiked with positive HPV calibrators1. The detection rates were:

- TP Vial workflow: 28% (95% CI, 9.7%-53.5%)
- APTIMA tube: 100% (95% CI, 81.5%-100%)

Utilising TP vials for storage, Daye Diagnostic tampons exhibited a sensitivity of 66.67% and specificity of 90.74% in HPV detection. Conversely, employing Aptima Multitest tubes for storage resulted in enhanced sensitivity and specificity of 83.33% and 85.42%, respectively. The sensitivity using Aptima Multitest tubes mirrored that of the Aptima Swabs, which reported a sensitivity of 84.6% (95% CI: 75.8—90.6%) in detecting high-risk HPV (hrHPV) (Hologic Inc., 2020).

For BV detection, Daye Diagnostic tampons exhibited 100.0% sensitivity and 96.43% specificity when stored in TP vials, whereas the sensitivity and specificity were 88.89% and 98.04%, respectively, when stored in Aptima Multitest tubes, compared to HCW-taken Aptima swabs' sensitivity of 95% (95% CI: 93.1—96.4%) (Hologic Inc., 2020). The sensitivity of CT and NG detection with the Daye Tampon in both TP Vial and Aptima tube was consistent with previous studies conducted with the Aptima Swab, showing sensitivity of 100% for both the TP Vial and Aptima Tube and a specificity of 100% and 98.31% for the TP Vial and Aptima tube, respectively (Hologic Inc., 2020).

These results highlight the potential of self-collected tampons as a robust diagnostic tool for HPV and STIs. Their comparative efficacy with HCW-obtained swabs positions them as a feasible alternative in scenarios with limited access to healthcare professionals.

The study's encouraging results are in line with previous research, which has demonstrated the feasibility and reliability of self-collected tampons for HPV and STI testing. Notably, Adamson et al. [1] and Tiiti et al. [32] reported similar findings regarding the comparability of self-collected tampons to traditional collection methods for HPV detection. Additionally, Bakkum-Gamez et al. [11] showed that tampons yielded sufficient DNA quantities and quality for endometrial cancer detection. Leveraging tampons as a collection device offers advantages, as they are familiar to many women and may reduce barriers related to embarrassment and fear when seeking healthcare.

In addition to evaluating diagnostic accuracy, an assessment of the acceptability and preference for the tampon self-collection method among participants was conducted. Notably, a substantial majority of participants (90%) exhibited a marked preference for the tampon self-collection approach over the HCW-collected swab method. This notable preference was primarily attributed to the perceived advantages of enhanced privacy and flexibility during the sample collection process. This suggests that the tampon self-collection method is well-accepted and feasible, especially among women already familiar with using tampons.

Employing self-collected tampons for HPV and STI testing offers several potential advantages. It is a non-invasive, cost-effective, and convenient method that can be performed at home, reducing the need for clinic visits and increasing screening accessibility. Self-sampling also overcomes emotional and practical barriers associated with traditional cervical cytology or HCW-collected HPV testing. These favourable outcomes support the inclusion of self-collected tampons as an alternative method for HPV and STI screening, particularly in low-resource settings where access to healthcare services is limited.

Our study has several limitations that should be considered when interpreting the results. The relatively small sample size and short study duration of three months may affect the generalisability of our findings. Large-scale studies conducted over longer periods would provide more robust evidence.

One of the main limitations of the study is the reduced sample size. This limitation, combined with the low prevalence of sexually transmitted infections in our study population, makes it difficult to assess the real analytical specificity and sensitivity of the assays and their implications in clinical practice. Since most of the women enrolled were negative for sexually transmitted pathogens, the study's ability to accurately determine the diagnostic performance of the tampon-based sampling method is limited.

Another limitation of this study was the lack of standardisation for sample volume across different collection methods. Tampons, due to their absorbent nature, require a larger volume of vaginal fluid compared to swabs for fixation. We acknowledge that this volume difference makes direct comparisons between collection methods challenging. Future studies should implement rigorous sample volume standardisation protocols, such as quantifying and normalising DNA yield or using a fixed elution volume across all sample types. This standardisation would ensure more accurate comparisons between tampon-based collection and traditional swab methods, providing a clearer picture of each method's true efficacy in detecting viral and bacterial pathogens in vaginal samples.

Future studies with larger, more diverse populations and higher prevalence of sexually transmitted infections are needed to fully assess the clinical implications of tampon-based sampling. These studies should aim to include a more representative sample of the target population, with a sufficient number of positive cases to allow for a more reliable evaluation of the assays' specificity and sensitivity.

Since this exploratory piece of research was conducted, we have conducted a larger study of the Daye Diagnostic tampon ($n=260$), which has validated results, confirming the novel collection device to have comparable diagnostic accuracy to clinician-collected vaginal swabs in detecting HPV [22].

Our study population consisted of participants recruited through female-focused social networks, which may introduce potential selection bias. To ensure representativeness, future studies should aim for more diverse populations, including individuals from various age groups, socio-economic backgrounds, and geographical locations.

The acceptability assessment relied on self-reported measures, which may be subject to recall bias or social desirability bias. Incorporating objective measures or qualitative interviews could offer deeper insights into the acceptability of the tampon-based sampling method.

While our study provides promising initial results, further research is necessary to validate the efficacy and clinical implications of tampon-based sampling for STI testing in larger, more diverse populations with a higher prevalence of infections.

Conclusion and recommendations

This study demonstrates that self-collected tampons show comparable diagnostic accuracy to HCW-collected swabs for HPV and STI detection. The tampon self-collection method is well-accepted and preferred by participants, indicating its potential as an alternative screening tool, particularly in low-resource settings. However, further research with larger sample sizes and diverse populations is warranted to validate these findings and inform the implementation of tampon-based self-collection programs for cervical cancer screening.

Enhancing generalisability and validation

To enhance the generalisability of the study findings, future research should ensure an adequate sample size and include individuals from diverse age groups, socio-economic backgrounds, and geographical locations. Randomized controlled trials (RCTs) should be considered to establish cause-and-effect relationships and minimize biases. Comparisons with gold standard methods, such as cervical cytology or physician-collected HPV testing, would further validate the efficacy of the diagnostic tampon.

Assessing new biospecimens in disease-prevalent contexts

When assessing new biospecimens like tampons to detect clinically relevant HPV infection, the common approach, as per international practices and guidelines, is to carry out this assessment in a context where the disease is prevalent, usually through colposcopy. In this method, the tampon's performance is measured against a well-established biospecimen, like liquid-based cytology, in detecting high-grade cervical lesions confirmed through colposcopy and sometimes biopsies. Studying new methods in a colposcopy setting or a disease-enriched context provides a quicker way to determine if the method is sensitive enough. Additionally, women who test positive for HPV in this setting are already undergoing colposcopy, ensuring their infection is appropriately managed following the standard medical pathway.

Public health implications and recommendations

The findings of this study have significant implications for public health, particularly in expanding access to cervical cancer and STI screening. Self-collected tampons present a viable alternative to traditional HCW-collected swabs, especially in LMICs where healthcare resources are limited. This method can be instrumental in overcoming barriers to screening, such as limited healthcare infrastructure, social stigma, and personal discomfort with HCW-collected samples.

Governments and health agencies, particularly in LMICs, should consider incorporating self-collected tampons into national cervical cancer screening programs to significantly increase screening rates, particularly in remote or underserved areas. Clear guidelines and protocols need to be developed for the use of self-collected tampons in HPV and STI testing, including instructions for collection, storage, and transport of samples to ensure accuracy and reliability. Public health initiatives should focus on educating women about the importance of regular screening and the availability of self-collection options.

Our study aligns with global health goals of enhancing healthcare equity, particularly for women in LMICs. Self-collected tampons can play a crucial role in reducing the incidence and mortality of cervical cancer in these regions by providing a more accessible and acceptable screening method. The implementation of self-collected tampons for screening could lead to more efficient use of healthcare resources, allowing reallocation of resources to other areas of need and potentially freeing up funds for other public health initiatives.

Ongoing research and future directions

While our study provides promising results, ongoing research is necessary to further validate the efficacy of tampons in different populations and settings. Research into the development of more sensitive and specific assays for use with tampon-collected samples could further improve the effectiveness of this screening method.

In conclusion, the use of self-collected tampons for HPV and STI screening has the potential to transform cervical cancer screening, particularly in low-resource settings. This study's findings should serve as a catalyst for policy changes and public health initiatives focused on reducing the global burden of cervical cancer.

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Authors' contributions

This manuscript represents a collaborative effort by Valentina Milanova, Iva Lazarova, Kalina Mihaylova, Michelle Gomes, Teodora Georgieva, and Jan Multmeier. The contributions of each author are outlined as follows: 1. Conceptualisation and Design of Study: This includes the initial idea, hypothesis formulation, and overall design of the study. VM, IL, KM 2. Data Collection and Analysis: Involves the gathering of data, either through experiments, surveys, or data mining, and subsequent analysis. VM, IL, KM 3. Writing—Original Draft Preparation: Refers to the primary creation of the manuscript text, including drafting the introduction, methodology, results, and discussion sections. VM, IL, KM 4. Writing—Review & Editing: Entails critically revising the manuscript for important intellectual content, including grammar, clarity, and organisation. VM, IL, KM, MG, TG 5. Visualisation and Figure Preparation: Includes the creation and design of figures, tables, and other visual elements to represent data and concepts. VM, IL, KM 6. Supervision and Project Administration: Overseeing the project, coordinating between team members, and managing timelines and resources. VM, IL, KM, MG 7. Funding Acquisition: Responsibility for securing the financial support for the project. VM Statistical Analysis and Interpretation: Involves applying statistical methods to analyse data and interpreting the results in the context of the study. JM 8. Ethical Approval and Patient Consent (if applicable): Responsibility for ensuring ethical compliance and obtaining necessary approvals and patient consents.VM, IL, KM 9. Critical Revision for Important Intellectual Content: Providing significant suggestions for improvement and refinement of the manuscript. MG, TG 10. Final Approval of the Version to be Published: Involves giving the final approval for the manuscript to be published after all revisions and editing. MG.

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Data availability

Data is provided within the manuscript or supplementary information files. Raw Data, can be provided on request. Please contact: Michelle.gomes@yourdaye.com.

Declarations

Ethics approval and consent to participate

This study was conducted in compliance with ethical guidelines and principles established by the ICH Guideline for Good Clinical Practice & Declaration of Helsinki and Bulgarian Health Law. The research protocol was reviewed and approved by Ascendent Medical Center, Bulgaria before data collection commenced. All necessary measures were taken to ensure the protection of the rights, dignity, and well-being of the participants involved.

Prior to participation, informed consent was obtained from all individuals included in this study. Participants were provided with a detailed explanation of the study objectives, procedures, potential risks and benefits, and their right to withdraw from the study at any point without consequences. Furthermore, they were assured of the confidentiality and anonymity of their personal information.

To protect the privacy and confidentiality of the participants, all data collected during this study was coded and stored securely. Any identifying information was removed or anonymized to maintain the privacy of the participants. Only authorised researchers had access to the data, and the information obtained was solely used for the purposes of this study.

This research adheres to the principles outlined in the ICH Guideline for Good Clinical Practice & Declaration of Helsinki. We recognise the importance of ethical considerations in research and strive to conduct studies that contribute to knowledge while respecting the rights and well-being of the participants involved.

Consent for publication

Annes Day LTD consents to the publication of the study "Efficacy and Acceptability of Self-Collected Medical Grade Tampon as a Novel Tool for Vaginal Sample Collection Tool for the Detection of HPV and STIs."

Competing interests

The authors declare no competing interests.

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