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Screening for High-Risk Human Papillomavirus Using Passive, Self-Collected Menstrual Blood

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OBJECTIVE: To assess concordance and acceptability of a modified menstrual pad compared with a clinician-collected high-risk human papillomavirus (HPV) sample.

METHODS: This was a prospective observational study. Women presenting for either cervical cancer screening or with a history of high-risk HPV positivity were eligible. Three samples were requested from participants: 1) clinician-collected cervical specimens; 2) self-collected vaginal swabs; and 3) a modified menstrual pad, which was taken home for use during the next menstruation. All samples were processed using the Cobas HPV test. Menstrual pad dried blood spots were eluted, then similarly processed.

RESULTS: Of 153 women enrolled in the study, 106 provided menstrual pad samples and clinician-collected cervical specimens for high-risk HPV analysis. For samples in which the interval between the clinician-collected specimen and the menstrual pad sample was less than 2 months, the concordance was 94% (95% CI 83–98). For women who tested positive for high-risk HPV who

presented for general screening and those with more than cervical intraepithelial neoplasia 2, menstrual pad and clinician-collected specimen agreement was 100% (95% CI 32.5–100). Among participants, 22.9% expressed discomfort with the self-collected vaginal swabs and opted out of collection. Overall, 94.0% of participants preferred the menstrual pad over clinician-collected sampling. Twelve patients were found to be positive for HPV on the menstrual pad sample but negative on the clinician-collected specimen.

CONCLUSION: Among women who tested positive for HPV, the menstrual pad showed highly concordant results compared with clinician-collected sampling. This collection approach shows promise for integration into cervical cancer prevention programs.

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Cervical cancer is the fourth most common cancer among women worldwide and the second highest cause of women's cancer-related mortality.¹ Most cases of cervical cancer are preventable through the combination of primary prevention, screening, and access to appropriate treatment of precancerous lesions.² Pap test cytology screening has been the most widely applied screening method for cervical cancer or precancer, but it has variable sensitivity and specificity and, globally, cytology is not effectively available to women in developing countries (where 80% of all cervical cancers occur).³ The combination of variable test qualities and limited access results, in part, in continued high incidence of fatal cancers in many settings. More than 50% of cervical cancers are diagnosed in patients who have not received screening in the previous 5 years.⁴

In recent years, tests to detect human papillomavirus (HPV), particularly high-risk subtypes, from cervical samples have been widely introduced and are rapidly becoming an accepted primary screening

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Sara Naseri is employed by Qurasense Inc., which manufactures the Q-Pads used in this study. Paul D. Blumenthal received payment from Bayer. The other authors did not report any potential conflicts of interest.

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approach.⁵ High-risk HPV screening is conventionally performed in the clinic and requires women to undergo a gynecologic examination, which is invasive, uncomfortable, and resource-intensive.⁶ High-risk HPV detection through self-collected samples could increase screening access while reducing both opportunity costs and uncomfortable examinations.⁷ A variety of self-collection devices have been used to detect HPV, including swabs, brushes, cervical lavage devices, tampons, and urine.⁸ Although most of these devices have comparable sensitivity for high-risk HPV (compared with clinician-collected samples),⁹ logistical challenges limit their utility. Most devices require instructions on proper insertion into the vagina (which can sometimes be culturally challenging), and the specimens are difficult to transport and often require storage in expensive, toxic, flammable fixative solutions.⁸ A novel and innovative alternative involves the use of menstrual blood for the same diagnostic purpose. To this end, a specialized collection device integrated into an ordinary, inexpensive menstrual pad has been developed.

The Q-Pad® (Qvin™, Menlo Park, CA) is a modified menstrual pad containing a paper-based dried blood spot strip, which enables convenient, noninvasive acquisition and stabilization of menstrual blood specimens. Such a collection device does not require intravaginal manipulation of a brush or swab or lavage device and could improve participation in community screening efforts while also being easier to transport than urine or cervicovaginal samples. Menstrual blood, whether collected by a menstrual cup or a modified menstrual pad, has already been shown to correlate well with a number of commonly used serum tests, such as hemoglobin A_{1C} and thyroid-stimulating hormone.^{10,11} Additionally, dried blood spot is arguably easier to transport than slides, urine, blood from a menstrual cup, or liquid-based cervico-vaginal samples; it requires no refrigeration, is not considered a hazardous material, and can potentially provide a variety of laboratory tests in a self-collected sample. Proteomic and small-scale HPV-detection studies on menstrual blood have been published recently,^{12–14} but rigorous, larger scale, and implementation-level studies remain to be performed.

We hypothesized that screening for cervical intraepithelial neoplasia (CIN) using self-collected menstrual blood, specifically using the Q-Pad, would compare favorably with currently implemented approaches, with high correlation to conventional high-risk HPV testing. We also hypothesized that it would be acceptable to women and possibly preferred compared with conventional examinations. We undertook this study to test these hypotheses.

METHODS

This was a prospective observational study of reproductive-aged women. The research protocols were approved by the Stanford University Institutional Review Board. All participants consented to the study and were compensated with a gift card on completion of the study protocol.

Participants were recruited through the gynecology clinic at Stanford University Medical Center (NCT03638427). For this convenience sample, women presenting for general screening were recruited. We included women with and without a history of high-risk HPV infection. Inclusion criteria included people older than age 18 years with a cervix who had regular menses. Individuals with postmenopausal status and pregnancy were excluded. We recorded basic demographic information and high-risk HPV status.

During the clinic visit, the participants had a clinician-collected cervical sample taken. Participants were also asked to perform a self-collected vaginal swab with guidance from study staff. In addition, all participants were issued a study kit containing two Q-Pads for menstrual blood self-collection at home. The Q-Pad is a modified conventional organic cotton menstrual pad containing a removable dried blood spot strip (Fig. 1). After use, the small collection strip is removed from the pad and the remaining pad is disposed of conventionally. The dried blood spot strip is placed in a sealed envelope and mailed to a laboratory using standard equipment for high-risk HPV analysis.

Participants were instructed to self-collect menstrual blood with the Q-Pad on the second day of their menstrual cycle, which generally corresponds with the

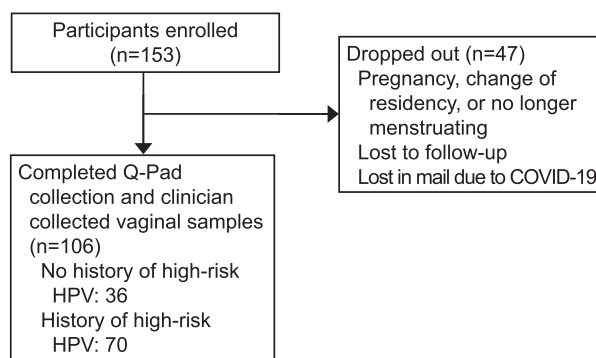


Fig. 1. Study design. COVID-19, coronavirus disease 2019; HPV, human papillomavirus.

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highest volume of flow. Once saturated, the participant placed the dried blood spot strip in a sample return box provided with the kit, which was put in a prepaid envelope that the study participants mailed back to the research team. All samples were then stored at ambient temperature (dried blood spot samples require neither refrigeration nor other means of preservation) before shipment to a reference laboratory (TriCore Labs) for analysis.

All samples (clinician-collected, self-swabs, and menstrual pad) were analyzed on the Roche Cobas 4,800 instrument. On laboratory receipt, all samples were logged and stored at room temperature. Menstrual pad strips were stored inside the return sample container used for shipping. The clinician-collected specimens were stored in PreservCyt liquid collection vials. Self-collected vaginal swabs were stored in the Cobas media solution. The time during which samples were stored at room temperature was variable, but, on average, samples were stored at room temperature for 10 days. Viral DNA has been shown to be stable in dried blood spot samples at ambient temperature for up to 52 weeks.¹⁵

For the self-collected vaginal swab and the clinician-collected samples, 1 mL of PreservCyt liquid or Cobas media solution was removed using a pipette and loaded into the tubes for the Cobas 4,800 analyzer. For the modified menstrual pads, a 6×6-mm punch was aseptically removed from the Q-strip and placed into a tube containing 1 mL AmpliPrep/Cobas TaQMan specimen pre-extraction reagent. The tubes were placed on a rack and put into a 37±2°C incubator for a total of 30 minutes and manually shaken every 10 minutes. All tubes were capped and bar-coded. Tubes were left overnight at 2–8°C and loaded onto the instrument the next morning. All tubes were run within 24 hours.

For the primary outcome of high-risk HPV results, concordance values with 95% CIs were calculated using GraphPad Prism using Wilson/Brown statistical methods for agreement of high-risk HPV results among clinician-collected specimens, self-collected vaginal swabs, and modified menstrual pad sample results. For sample sizes of seven or less, CIs were calculated using the Poisson distribution. Kappa statistics were derived from these data. Missing data were excluded from the analysis and related only to demographic variables. Demographic attributes of the total recruited group and the evaluable group were compared using χ^2 test. Information on race and ethnicity was included to be informative about the nature of our population and to demonstrate that the overall recruited and the evaluable groups were not skewed in any racial or ethnic direction.

RESULTS

A total of 153 volunteers were enrolled between December 2019 and February 2021. Clinician-collected cervical specimens and at-home self-collected menstrual blood were obtained from 106 participants (Fig. 2). Among this cohort, a total of 34% had no previous history of high-risk HPV infection. The mean age at enrollment was 32.2 years (Table 1).

In the cohort of women with no previous history of high-risk HPV infection who came in for general screening (n=36), the percent agreement between clinician-collected cervical specimens and at-home self-collected menstrual blood for participants who tested positive for high-risk HPV was 100% (Table 2). Among participants who were enrolled in our study at the time of a previously scheduled colposcopy and had a biopsy taken that showed CIN 2 or CIN 3 (n=5), the percent agreement between clinician-collected cervical specimens and at-home self-collected menstrual blood was also 100%.

Among women who had a clinician-collected specimen and then used the modified menstrual pad within 2 months, the concordance among those with a positive clinician-collected cervical specimen was 93.5% (95% CI 82.5–97.8). For the self-collected vaginal swab (which was collected at the same clinic visit as the clinician-collected swab), the same concordance with the clinician-collected specimen was 88.1% (95% CI 75.0–94.8). This yields a Cohen's kappa statistic of 0.72 for the modified menstrual pad compared with the clinician-collected swab ($P<.001$), indicating substantial agreement.

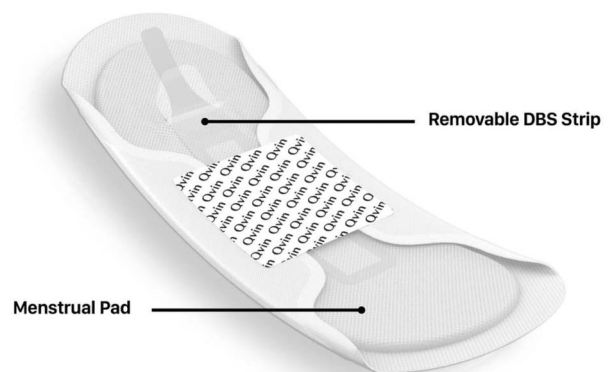


Fig. 2. Composition of the Q-Pad, a modified menstrual pad made from 100% organic cotton with an embedded, removable dried blood spot strip. Image courtesy of Qvin. Used with permission.

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Table 1. Participant Characteristics

Characteristic	Overall (N=153)	Evaluable Group (n=106)	P
Age (y)	32.2±6.7	31.0±6.9	.163
Minimum–maximum	21–49	21–49	
Parity			.602
0	122 (79.7)	85 (80.2)	
1	13 (8.5)	10 (9.4)	
2	10 (6.5)	11 (10.4)	
3	3 (2.0)	3 (2.8)	
4	3 (2.0)	0 (0.0)	
Missing data	2 (1.3)	4 (3.8)	
Ethnicity			.616
Hispanic, Latina	22 (14.5)	13 (12.4)	
Non-Hispanic, Latina	129 (85.4)	92 (87.6)	
Missing data	2 (1.3)	1 (0.9)	
Race			.996
Asian	54 (35.5)	38 (35.8)	
Black	4 (2.6)	2 (1.9)	
More than one race	2 (1.9)	1 (0.9)	
Native Hawaiian/Other Pacific Islander	2 (1.32)	2 (1.9)	
White	72 (47.7)	51 (48.1)	
None of the above	18 (11.8)	12 (11.3)	
Missing data	1 (0.1)	0 (0.0)	

Data are mean±SD or n (%) unless otherwise specified.

Due to reduced clinical operations and lockdowns of various durations related to the coronavirus disease 2019 (COVID-19) pandemic, 12 participants were unable to provide both samples within our anticipated 2-month interval. For these patients, the average interval between collected samples was 5.6 months. If these cases are included in the analysis, the concordance between clinician-collected specimens and modified menstrual pad results was 87%. However, none of these patients had evidence of high-grade disease. This analysis was not done for self-collected vaginal swabs because these were collected at the same time as the clinician-collected swab. Finally, for 12 participants, the modified menstrual pad detected high-

risk HPV and the clinician-collected sample did not.

Ninety-four percent of participants (78/83) said they would prefer the modified menstrual pad over clinician-collected samples if it were available as an alternative to high-risk HPV screening. Ninety-two percent of participants said they preferred self-collection methods over clinician-collected screening, and, of these, the vast majority (94%) stated that they preferred the modified menstrual pad passive at-home collection over the self-collected vaginal swab. Just under twenty-three (22.9) percent of participants opted out of the self-collected vaginal swab altogether because they felt uncomfortable with the procedure. No participants expressed discomfort with the modified menstrual pad.

Table 2. Clinical Outcomes

Agreement Between Modified Menstrual Pad or Self-Collected Vaginal Swab and Clinician-Collected Specimen	Percent Agreement (95% CI)
Modified menstrual pad vs clinician-collected specimens	
In those with moderate-to-severe dysplasia (CIN 2 or worse) (n=5/5)	100 (32.5–100)
In the general population with high-risk HPV–positive results or in those with moderate-to-severe dysplasia (CIN 2 or worse) (n=7/7)	100 (40.2–100)
For high-risk HPV–positive specimens collected within 2 months (n=43/46)	93.5 (82.5–97.8)
Self-collected vaginal swabs vs clinician-collected cervical specimens with high-risk HPV–positive results (same day as the clinician-collected specimen) (n=37/42)	88.1 (75.0–94.8)
Patients who preferred the modified menstrual pad to clinician-collected cervical specimens (n=78/83)	94.0
Patients who opted out of self-swab due to discomfort with the procedure (n=32/141)	22.9

CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus.



DISCUSSION

We compared the performance of at-home self-collected menstrual blood, in-clinic self-collected vaginal swabs, and clinician-collected specimens for high-risk HPV detection. Menstrual blood was obtained using a novel, noninvasive collection device, the modified menstrual pad, which generates a stable, transportable dried blood spot specimen from menstrual blood. There was a high correlation for high-risk HPV-positive detection (93.5%) between self-collected modified menstrual pad samples and clinician-collected specimens. This was higher than the concordance for the self-collected vaginal swabs. This, combined with almost one quarter of women expressing discomfort performing a self-collected vaginal swab shows promise for at-home cervical cancer screening performed using passively collected menstrual blood with the modified menstrual pad. A public health intervention such as cervical cancer prevention ultimately requires testing approaches that are convenient and expeditious and allow for easy community penetration to achieve high coverage. Menstrual blood offers noninvasive access to blood and endometrial tissue, which can be collected by patients in the privacy and comfort of their home, using a menstrual collection pad with which most women are both familiar and comfortable. For developing countries, similar to experiences with careHPV in Thailand,¹⁶ this approach could also be used in field settings to which a woman could bring her menstrual pad strip, with point-of-care test results possible.

This study has several limitations. We observed an unanticipated time lag among many participants between the clinician-collected specimen and the modified menstrual pad return due to the COVID-19 pandemic and its effect on clinical operations, including lockdowns of various durations. As a result, a number of participants were unable to provide both samples within our anticipated 2-month interval. This had an effect on the percent concordance between the two methods. In total, only seven samples were discordant between the menstrual pad and clinician-collected samples (where the latter was HPV-positive), but for four of the seven, multiple cycles (months) passed between the clinician-collected samples and modified menstrual pad use. In one instance, more than 10 months passed. Due to the nature of high-risk HPV, it is at least conceivable that the virus had cleared during that interval. Indeed, given the relatively high correlation between concurrent and short-interval collections, the decrease in concordance might represent HPV clearance from the system. This is supported by the fact that all but one of these women

were young (27–32 years old), a group among whom viral clearance is known to occur.¹⁷ To confirm that the virus had cleared, it would have been desirable to perform another Pap test at the time of modified menstrual pad use, but, due to the COVID-19 pandemic, it was not possible for study staff to ask women to come back to the clinic for another clinician-collected sample. Nor was it possible to ask women to return to the clinic for a confirmatory colposcopy or biopsy for study purposes. Ensuring minimal lag times between clinician-collected specimens and modified menstrual pad return will be an important requirement in a future validation study.

Another limitation to the study relates to those cases in which the modified menstrual pad was positive for high-risk HPV but the clinician-collected sample was not. In the majority of such cases (8/12), high-risk HPV was also not detected on the self-collected vaginal swab. In the four cases in which the self-collected vaginal swab detected high-risk HPV, it is reasonable to consider that the high-risk HPV detected may have resided in the vaginal canal or on the vulva. The same could be true for the modified menstrual pad. For the eight cases in which the modified menstrual pad detected high-risk HPV but the self-collected vaginal swab and the clinician-collected specimen did not, it is feasible that the high-risk HPV resided higher up in the cervix, where neither the clinician nor the self-collected vaginal swab reached but through which menstrual blood still flowed. In essence, it is possible that this could represent a sampling error for the clinician-collected or self-collected vaginal swab specimens, because it is very unlikely that the COBAS analyzer would return a positive high-risk HPV result when no high-risk HPV was present. This could especially be likely among nulliparous women, because an endocervical sample may be less easily obtained than among parous women, which was the case for most of these participants (9/12). Another explanation could be that the patients contracted high-risk HPV between samples collected, but, because all but two of these samples were collected in the same cycle, the likelihood of these patients contracting high-risk HPV between samples collected is less likely. Finally, perianal infection is also possible, but, given the mechanics and design of the modified menstrual pad, this is unlikely and yet could not be assessed in this study. These data also indicate that, for a study such as this, the clinician-collected swab cannot be considered a true reference standard because the apparent discrepancy may be due to sampling error. This could be resolved by obtaining a specimen representative of the entire cervix for viral cultures, but that was not clinically feasible for this project.



Another limitation to this study was that the method of the modified menstrual pad analysis was not optimized. Because this was the first attempt to isolate high-risk HPV from a dried menstrual blood spot, test procedures may not have been optimized for this approach. Future studies will be required to determine whether and how tests derived from menstrual blood can be performed for improved, optimal results.

Finally, a number of women enrolled but did not complete the study. In many cases, lack of completion was due to COVID-19-related exigencies, such as patients not being allowed back into the clinic for research examinations and lack of institutional postal service for several months during the pandemic. However, the demographic profile of those who did complete the study is highly concordant with the total group enrolled, so a different outcome on these results seems unlikely.

Sample collection using the modified menstrual pad can provide a simple self-collection option with personalized results to predict the individuals at highest risk and triage them to care. This could increase the ease of screening (and therefore screening participation) for women and decrease unnecessary office visits and procedures. Screening for cervical high-risk HPV using self-collected menstrual blood with the Q-Pad demonstrated high correlation (94%) with conventional high-risk HPV testing. These results are very similar to a study recently published by Zhang et al,¹⁸ in which a conventional menstrual pad was used for analysis (ie, no strip containing the dried blood spot) with highly comparable results.

Importantly, women found using the modified menstrual pad both highly acceptable and preferable to conventional high-risk HPV specimen acquisition with speculum examination. This approach to screening may also allow for improved access to screening for cancer and precancer in the areas with the highest burden of disease, including unscreened women in the United States and worldwide. Although this passive collection approach shows promise for integration into cervical cancer screening programs, larger studies will be required for more definitive validation.

The potential applications of this collection approach could also include other critically important biomarkers such as for ovarian, endometrial, and other cancers, as well as sexually transmitted infections. It therefore has the potential to be used for highly personalized, self-collected screening for a variety of issues of public health importance in both high-resource and low-resource settings.

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