RESEARCH ARTICLE

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Human Papillomavirus Genotype Detection and Cytology Using a New Self-Sampling Method

Hiromi Yabusaki*, Midori Ono, Natsuko Shiina, Yoshio Shiina

Abstract

Objective: High-risk human papillomavirus (hrHPV) testing using dry-type self-sampled vaginal specimens is becoming more widespread worldwide due to increased screening uptake. However, for the triage of hrHPV-positive women, a visit to a general practitioner is required for reflex cytology. This study aimed to evaluate the hrHPV detection capability of CellSoft®, a wet-type self-sampling method that also allows for cytology. **Methods:** Thirty-eight women aged 20 years and older were included in the study. The women self-sampled using CellSoft® after using an Evalyn® Brush. PCR-based HPV genotyping was performed on both specimens and hrHPV detection results of both devices were compared. Additionally, cytological exam was performed on CellSoft® samples. **Results:** Overall agreement between self-sampling devices for the detection of hrHPV in CellSoft® and Evalyn Brush was observed in 97.4% (37/38) of participants. More hrHPV genotypes were detected with Evalyn Brush than with CellSoft®. Among the 22 CellSoft® hrHPV-positive cases, 11 (47.6%) were atypical squamous cells of undetermined significance or worse. **Conclusion:** CellSoft® hrHPV genotype detection results were in good agreement with those of Evalyn Brush. CellSoft® provided a sufficient cell volume for HPV testing and cytological evaluation.

Keywords: Self-sampled device- CellSoft®- Evalyn® Brush- High-risk Human Papillomavirus (hrHPV)

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Introduction

High-risk Human Papillomavirus (hrHPV) testing is the primary method of cervical cancer screening [1, 2]. However, solving the problem of low screening rate remains challenging. In Japan, the rate of cervical cancer screening uptake is as low as 40% [3]. Many factors deter women from participating in screening. The factors include feeling embarrassed to undergo the gynecological exams, fear of pain and the results, and time constraints [4]. Therefore, vaginal self-sampling for hrHPV testing has been proposed as an additional strategy to encourage women to take screening tests [5-7].

The Evalyn® Brush, a self-sampling device, is widely used worldwide due to its suitability for mailing (as the device can be dried after collection) and its high performance as well as its patient acceptance [8, 9]. Accumulating evidence suggests that the detection rates of hrHPV and cervical intraepithelial neoplasia (CIN) 2+ in these self-sampled specimens are comparable to those of physician-collected specimens [10-12]. Therefore, it is possible for self-sampled specimens to be the preferred option for increasing the number of primary screening tests.

On the other hand, there is concern that the proportion of patients undergoing cytological triage after a self-sampled specimen is found to be hrHPV-positive is not high [13]. Since cytological triage after primary screening reduces cancer incidence and unnecessary colposcopy procedures [13, 14], the next challenge is to improve the proportion of hrHPV-positive women undergoing reflex cytology performed by their general practitioners.

CellSoft®, recently developed in Japan, is a self-sampling device for hrHPV testing that improves on Kato's self-sampling device [15-18] for cytology, which has gained high rate of acceptance among women, especially in East Asia. It is a tampon-like device with a wider cell collection sponge than other self-sampling devices, allowing it to collect a sufficient number of cells [16]. Consequently, 100% agreement between this self-collection technique and the collection by physicians has been reported in terms of specimen adequacy and the measurement of abnormalities in cytology interpretation cells [18]. Additionally, it can be mailed without drying, making it suitable for both HPV and cytology testing.

In this study, we aimed to evaluate the performance of CellSoft® by conducting a Papanicolaou (Pap) test on it in conjunction with a comparison of PCR-based HPV genotype detection results between CellSoft® and Evalyn® Brushes.

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Materials and Methods

Clinical specimen collection

Between May 2022 and June 2022, clinical specimens were collected from 38 women, including 30 commercial sex workers (CSWs) aged at least 20 years in Tokyo area and 8 female employees (FEs) at the Fukui Obstetrics and Gynecology Clinic. After accepting their informed consent, the women self-sampled vaginal specimens using the Evalyn® Brush (Rovers Medical Devices B.V., The Netherlands). Subsequently, additional specimens were collected using CellSoft® (Taisei Medical Co. Ltd., Osaka, Japan; Figure 1 and Figure 2) following the procedure presented in Figure 3. All specimens were transported at room temperature to the ILABO Cyto STD Laboratory (Tokyo, Japan) for HPV testing and cytology (CellSoft® only). Each specimen was assigned an anonymous unique patient code.

HPV genotyping

The CellSoft® sponge was washed with 5 ml of fixative solution containing 50% ethanol, and the sponge was squeezed to suspend adherent cells. One ml of the cell suspension was centrifuged at 3,000 rpm for five minutes to obtain cell pellet. DNA from the cell pellet was isolated using the hot sodium hydroxide method [19]. The cell pellet was lysed with 50 µL of alkaline lysis solution (25 mM NaOH and 0.2 mM ethylenediaminetetraacetic acid [EDTA]; pH, 12.0) for 30 min at 95°C. The lysed cells were then neutralized with 0.04 M Tris-HCl (pH 5.0), centrifuged at 13,200 rpm for 1 minute, and directly used as the DNA template for PCR amplification; the DNA template was not quantified. For Evalyn Brush, the brush was placed directly into a 1mL aliquot of an alkaline lysis solution to lyse the cells. HPV genotyping was performed using a highly sensitive PCR-based HPV genotyping known as uniplex E6/E7 PCR [20]. This method is capable of detecting 39 HPV genotypes from as few as 100 viral copies, with no cross-reactivity for all HPV genotypes. To ensure that there was no DNA contamination causing false positives, each round of PCR was performed with a negative control using DNase-free water.

Pap Test

After centrifuging the cell suspension at 3,000 rpm for five minutes, the supernatant was discarded and 1–2 drops of the sediment were dropped onto a glass slide. The sediment was then spread between the two glass slides using the pull-apart method. The smeared slides were completely dried first before fixing in 95% ethanol, and then stained with Papanicolaou's stain. Pap smears were evaluated for the following: 1. Specimen adequacy,

Figure 1. Appearance of Cellsoft. The device is shown with the sponge extruded for scraping

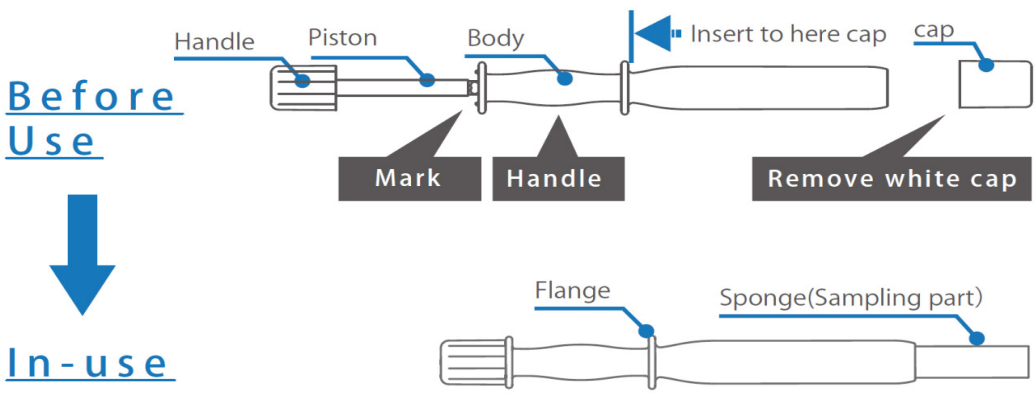

Figure 2. Structure of the Cellsoft Device

determined as either satisfactory or unsatisfactory upon slide evaluation, 2. The presence or absence of transformation zone components, requiring at least ten endocervical or metaplastic cells), 3. Cytological interpretation according to the Bethesda system [21].

Results

HrHPV genotype detection results with CellSoft, Evalyn Brush, and Papanicolaou test results with CellSoft are shown in Table 1. CellSoft detected hrHPV in 58.9% (22/38) of cases, while Evalyn Brush detected in 55.3% (21/38) of the cases. The hr HPV positivity rate of CSW was 73.3% (22/30) for CellSoft and 70.0% (21/30) for

Table 1. CellSoft and Evalyn Brush hrHPV Genotype Detection and Papanicolaou Test Results

	CellSoft				Evalyn Brush
	hrHPV genotype	Papanicolaou test			
		Specimen adequacy	Transformation zone component #	Cytological interpretation	hrHPV genotype
Commercial sex workers					
1	31,58	Satisfactory	Absent	NILM	31,51,58,59
2	58	Satisfactory	Absent	NILM	58
3	39,51	Satisfactory	Absent	NILM	39,51,58
4	58	Satisfactory	Present	NILM	Negative
5	Negative	Satisfactory	Present	NILM	Negative
6	58	Satisfactory	Present	NILM	58
7	52,58	Satisfactory	Present	NILM	52,58
8	51	Satisfactory	Present	NILM	51,58,59
9	Negative	Satisfactory	Present	NILM	Negative
10	Negative	Satisfactory	Present	NILM	Negative
11	52	Satisfactory	Present	NILM	52
12	39,59	Satisfactory	Absent	NILM	39,59
13	39	Satisfactory	Present	NILM	39
14	52,58	Satisfactory	.Absent	NILM	39,52,58
15	Negative	Satisfactory	Absent	NILM	Negative
16	52,56	Satisfactory	Present	NILM	56
17	Negative	Satisfactory	Absent	NILM	Negative
18	Negative	Satisfactory	Present	NILM	Negative
19	51,52,58,59	Satisfactory	Absent	ASC-US	51,52,56,58,59
20	Negative	Satisfactory	Absent	ASC-US	Negative
21	18,51	Satisfactory	Absent	ASC-US	18,51,58
22	52,58,59	Satisfactory	Absent	ASC-US	16,33,52,58,59
23	58	Satisfactory	Absent	ASC-US	58
24	31,33,39,51,52, 56,58,59	Satisfactory	Absent	LSIL	31,33,39,51,52, 56,58,59
25	Negative*	Satisfactory	Absent	LSIL	Negative*
26	51, 56,58,68	Satisfactory	Present	LSIL	51,56,58,68
27	51,56,59	Satisfactory	Present	LSIL	51,56,59
28	Negative*	Satisfactory	Present	LSIL	Negative*
29	45,56,58	Satisfactory	Present	ASC-H	45,56,58
30	16	Satisfactory	Present	HSIL	16
Female employees					
31	Negative	Satisfactory	Present	NILM	Negative
32	Negative	Satisfactory	Absent	NILM	Negative
33	Negative	Satisfactory	Absent	NILM	Negative
34	Negative	Satisfactory	Absent	NILM	Negative
35	Negative	Satisfactory	Present	NILM	Negative
36	Negative	Satisfactory	Absent	NILM	Negative
37	Negative	Satisfactory	Absent	NILM	Negative
38	Negative	Satisfactory	Absent	NILM	Negative

* hrHPV was negative but HPV53 was positive. #Presence of more than 10 endocervical or squamous metaplastic cells. hrHPV, high-risk type human papillomavirus; NILM, negative for intraepithelial lesions or malignancy; ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; ASC-H, atypical squamous cells that cannot exclude a high-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion

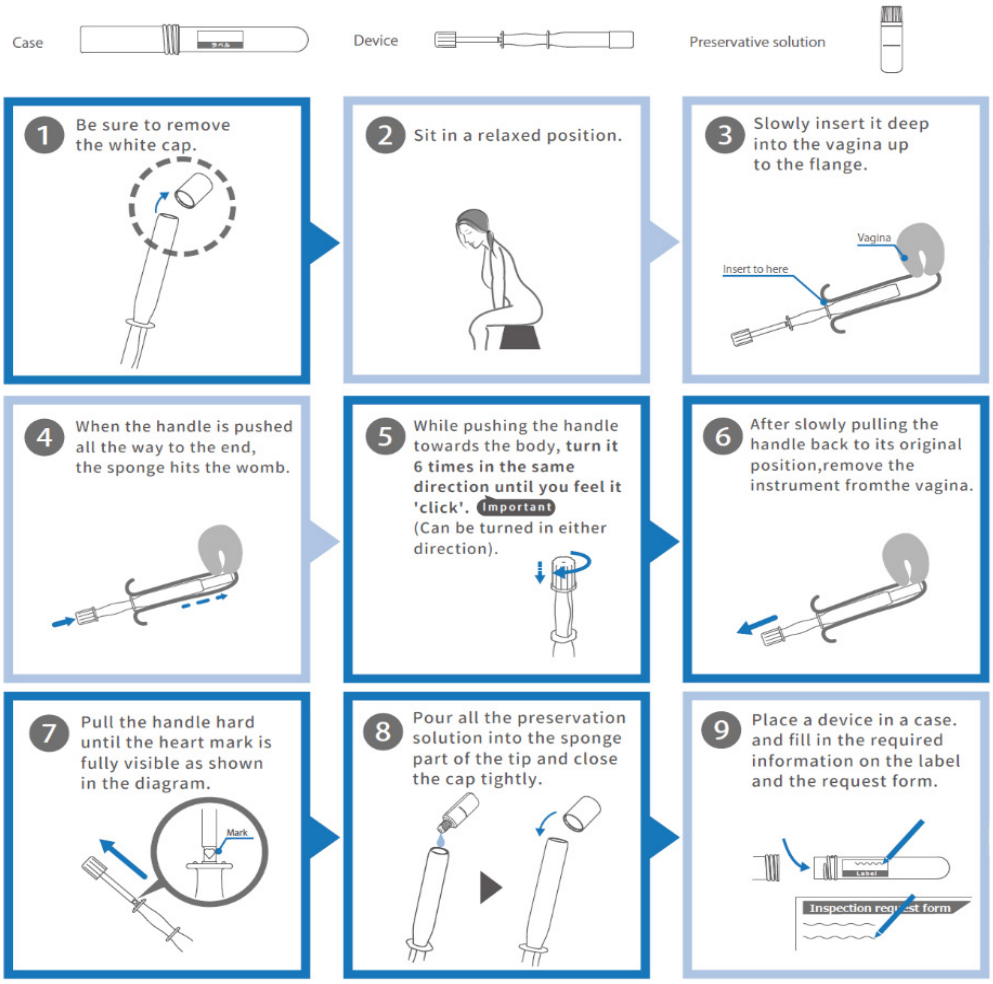

Figure 3. Female Self-Collection Procedure

the Evalyn Brush. No hrHPV was detected in samples collected from the FEs using either device. The overall agreement between both collection instruments for the detection of hrHPV was observed in 97.4% (37/38) of cases ($\kappa = 0.946$; $P < 0.0001$). Among the 22 cases that were HPV-positive with both CellSoft and Evalyn Brush, 7 (31.8%) had more HPV genotypes detected with Evalyn Brush than with CellSoft.

The evaluation of the Pap smears collected by CellSoft showed that all were adequate with sufficient cell volumes for diagnosis. Transformation zone components were also seen in 45% (17/38) of participants. Cytologic interpretation revealed that 31.6% (12/38) had ASC-US or worse, and the percentage was 40% (12/30) when limited to CSW only. Amonng the 22 hrHPV-positive cases in CellSoft, 11 (47.6%) had ASC-US or worse.

Discussion

Women in Japan who do not undergo cervical cancer screening remain at high risk of developing cervical cancer due to the lack of vaccination. Increasing their participation rates for cervical cancer screening is an important way to reduce the incidence of cervical cancer. Self-sampling can help reach out to this untested population [5-7]. The recently developed CellSoft® is a tampon-like self-sampling device. Unlike their Western counterparts, Japanese women have little experience with tampons or vaginal products, so they may be resistant to the idea of inserting a foreign object into the vagina. However, in a study of Japanese women, by Hanley et al. [9], it was found that compared to physician-assisted testing, women found self-sampling being significantly less painful and less embarrassing, creating a less stressful environment for cervical cancer screening tests. They also found that a history of tampon use was not a barrier to a person's willingness to undergo self-sampling again. Thus, self-sampling for hrHPV testing with CellSoft is also expected to become widely accepted in Japan.

The present study directly compared the performance of two self-sampling devices in detecting hrHPV in the same woman using a PCR-based HPV test [20] that has been validated to detect a wide range of HPV with high sensitivity.

When comparing the types of hrHPV genotypes detected, more genotypes were detected with Evalyn than with CellSoft. This is likely because the vaginal specimens collected with CellSoft were collected after self-sampling with Evalyn Brush. Nevertheless, there was good agreement between the CellSoft and Evalyn Brush samples in terms of hrHPV detection. CellSoft device has been shown through its development to collect sufficient

cells with fewer inadequate specimens, and much greater sensitivity to intraepithelial lesions compared to other self-sampling methods [16, 18]. In this study, all Pap smears evaluated were adequate in cell quantity with 45% of them showing cells in the transformation zone. This indicates that CellSoft can collect cells from a wide area of the vagina, including the cervix. One case was hrHPV-negative with Evalyn Brush and hrHPV-positive with CellSoft. Since hrHPV is known to preferentially infect the squamous junction of the cervix (SCJ) [22, 23], it was suggested that CellSoft was more likely to contact areas closer to the SCJ. However, many cervical cells naturally exfoliate and then float in vagina [24], so it is possible that CellSoft may have accidentally captured hrHPV-infected cells.

A meta-analysis of 20 reported follow-up compliance rates for women who self-sampled positive for hrHPV averaged 80.6% (95%CI: 67.0%–91.5%) [13], which is not necessarily high. Thus, if hrHPV-positive women visit their gynecologists and are not adequately triaged by reflex cytology, the overall improvement in cervical cancer screening rates may be partially undermined. While the use of self-sampled specimens for cytology is not recommended due to the low accuracy of cytological diagnosis [25], the value of triage by cytology using self-collected samples from hrHPV-positive women is unclear. In a recent prospective cohort study, Loopik et.al. [26] showed that reflex cytology using hrHPV-positive self-samples is applicable and has added value as a direct triage test for stratifying colposcopy referrals. Wiersma et al. [27] also found that more than 35% of women with abnormal reflex cytology using self-collected samples could be referred directly to colposcopy without the need for an additional Pap smear. While Evalyn Brushes are dried for transport and cannot be used for self-sampled cytology, CellSoft was originally designed for self-sampling cytology and can collect cells of similar quality and quantity to those collected by physicians [18]. Therefore, self-samples can be used for hrHPV testing initially, and cytologic diagnosis can be performed at a later date as needed.

In fact, all hrHPV-positive women in the study were CSWs, with a positivity rate of about 70%. Since the hrHPV positivity rate among sex workers is reportedly 8%–100% [28], which is several times higher than that of the general female population, more women should be triaged with Pap testing especially in this population. To emphasize this, about half of the HPV-positive women in this study were determined to have ASC-US or worse by Pap testing using CellSoft. Therefore, a single self-sample testing allowed those women to avoid additional cytology testing by a physician and to be referred directly to colposcopy. Although it cannot replace the performance of physician-collected cytology, if reflex cytology using self-sampling specimens provides information about atypical cells, it may improve patient satisfaction, and thus reduce diagnostic delays and the opportunity for follow-ups. Therefore, we believe that the wet-type self-sampling method should be reconsidered as an attractive alternative to improve the triage of reflex cytology in hrHPV-positive women.

Nevertheless, this study has some limitations that are worth mentioning. Our statistics and inferences were restricted by the limited number of samples included in this study. Unfortunately, physician-collected cell samples were not used in this study, so CellSoft could not be evaluated for cytological diagnosis compared to physician-collected specimens. Therefore, further studies are needed to evaluate the performance of this self-sampling method.

CellSoft showed good agreement with Evalyn Brush, which is known for high performance and patient acceptance as a self-sampling device, in hrHPV detection results. Additionally, CellSoft provides the added value of reducing the rate of loss to follow-up by offering reflex cytology without sampling again.

Author Contribution Statement

Conceptualization, Y.S.; methodology, H.Y. and M.O.; validation, Y.S. and H.Y.; formal analysis, M.O.; investigation, Y.S.; re-sources, Y.S.; data curation, Y.S.; writing-original draft preparation, Y.S.; writing-review and editing, Y.S.; visualization, M.O.; project administration, Y.S., All authors have read and agreed to the final version of the manuscript.

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General

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Ethical Declaration

Informed consent was obtained from all study participants before their inclusion in the study. The study protocol was approved by the ILABO Cyto STD Laboratory Research Ethics Committee and was conducted according to approved guidelines.

Data Availability

The data and materials that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interest Statement

Yoshio Shiina received support from Taisei Medical Co. Ltd for providing English proofreading. The other authors have no conflict of interests related to this publication.

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