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Vaginal self-sampling without preservative for human papillomavirus testing shows good sensitivity

Lotten Darlin[a,*], Christer Borgfeldt[a], Ola Forslund[b], Emir Hénic[a], Joakim Dillner[b,c,d], Päivi Kannisto[a,e]

[a] Department of Obstetrics and Gynaecology, Skane University Hospital, Lund University, Sweden
[b] Department of Laboratory Medicine, Medical Microbiology, Malmo Skane University Hospital, Lund University, Sweden
[c] Department of Laboratory Medicine, Karolinska Institute and Hospital, Stockholm, Sweden
[d] Department of Medical Epidemiology & Biostatistics, Karolinska Institute and Hospital, Stockholm, Sweden
[e] Department Obstetrics and Gynaecology, Gynaecologic Oncology, Kliniken-Essen-Mitte, Henricistrasse 92, D 45136 Essen, Germany

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ABSTRACT

Background: Several strategies have been used to reach non-attending women in organized cervical-cancer-screening programs, with varying success. Self-sampling (SS) for HPV is effective for increasing coverage in screening programs, but requires expensive commercial sampling kits.
Objective: We aimed to evaluate if vaginal SS, without commercial preservatives was adequate for HPV testing.
Study design: Women with abnormal cervical smears as determined from the organized screening program were invited to a colposcopy clinic. The 121 women were asked to insert a cotton swab into the vagina and rotate it, put the cotton swab into a sterile cryotube, break the upper part of the stick and put the cap on. Thereafter, the gynaecologist collected a liquid based cytology (LBC) sample. The presence of HPV-types in SS and LBC samples was analysed with PCR and luminex-based typing.
Results: High-risk-HPV (hr-HPV) DNA was found in 65 of the tested 108 SS (60%; 95% CI 0.50–0.69), whereas LBC found hr-HPV in 64/108 samples (59%; 95% CI 0.49–0.69). The agreement between sampling with SS and LBC was good, kappa value 0.67 (95% CI; 0.53–0.81). The sensitivity for SS with hr-HPV to find HSIL was 81% (95% CI; 67–95%), specificity 49% (95% CI; 37–60%) and the sensitivity for LBC with hr-HPV to find HSIL was 90% (95% CI 80–100%), specificity53% (95% CI; 42–65%).
Conclusions: This new vaginal self-sampling method detects hr-HPV-infections with similar sensitivity as a cervical smear taken by a gynaecologist. This self-sampling method is cost-effective and well tolerated, and the kit is suitable for regular mail transport.

1. Background

The national organized screening program to prevent cervical cancer was introduced in Sweden in the 1960s, and has decreased the incidence and mortality of cervical carcinoma.[1] In spite of this, almost 500 women are diagnosed with cervical cancer every year. The screening program covers approximately 80% of women in the screening ages of 23–65 years. There is international consensus that a well-organized screening program of the female population is a cost-effective method to find cervical dysplasia and to prevent cervical cancer. Almost half of the cervical cancer cases in Sweden develop in women who do not attend organized screening, or in women who have passed the organized screening program age.[2] About one third of the new cases occur in the screened population. They are usually less advanced or micro-invasive and are mainly curable by surgery.[3] Several strategies have been used to reach the non-attending women in the organized screening program, with varying success rates. Persistent infection with high risk human papilloma virus (hr-HPV) is the main reason for more than 99% of cases of cervical intraepithelial neoplasia, as well as for invasive cervical cancer.[4] It has been shown that vaginal smear analyses for the presence of hr-HPV-infection is a successful method to find individuals with a high risk of cervical cancer.[2,5–7] HPV-positive women have been invited to further investigation.

There are several different commercial vaginal self-sampling devices on the market, which are used to test for the presence of HPV infection.[8–11] However, most of them are either quite expensive or difficult to use or manage in the logistics. We present a new cost-effective sample method, which consists of a cotton-swab (Selefa Trade, Sweden) and dry sterile 2 mL plastic container

* Corresponding author at: Department of Obstetrics and Gynecology, Skane University Hospital, SE-221 85 Lund, Sweden. Tel.: +46 46 17 25 20; fax: +46 46 15 78 68.
E-mail address: Lotten.Darlin@med.lu.se (L. Darlin).

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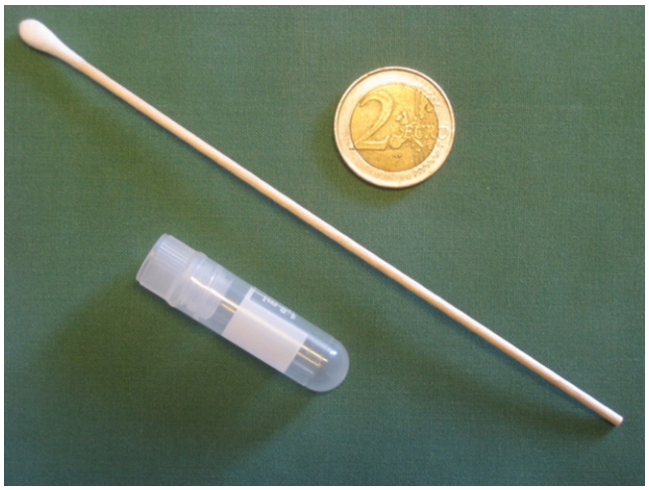

Fig. 1. Vaginal self-collected kit including sterile cotton swab and sterile cyto-tube with a screw cap. Total cost including mail transport approximately two euros.

(Cryotube, Nunc A/S, Denmark) (Fig. 1). No transport medium is needed, which makes it suitable for regular mail transport. The cost for the sterile cotton swab and the cryotube, including mail delivery is approximately two euros. The main purpose of the study was to investigate if the self-sampling described in this study has the same accuracy as the present standard method used for HPV analyses, which, in our region, has so far involved physicians taking cervical smears (LBC-Thin-Prep).[2,12,13]

2. Objectives

Self-sampling for HPV has been found to be effective to increase coverage in cervical screening programs, but requires expensive commercial sampling kits.[9,14] The aim was to evaluate if vaginal self-sampling, without preservatives was adequate for HPV-testing.

3. Study design

One hundred and twenty-one women, aged 18–65 (mean 34 years), who had been found to have an abnormal cervical smear in the organized screening program, were invited to the outpatient colposcopy clinic at Lund University Hospital. Tests from 108 women who had given informed consent were analysed. Oral and written instructions were given to the study persons before taking the self-collected vaginal sample (SS). Briefly, the women were asked to place the cotton swab 6–10 cm up into the vagina and rotate it 360 degrees 3–4 times. They then put the wooden stick into a sterile cryotube, broke the upper part of the stick and put the cap on. Immediately thereafter the consultant collected the standard liquid based cytology (LBC) for HPV detection from all the patients.

The LBC was collected by a plastic device Rovers® CervexBrush® Combi scraping cells from portio and put into a "Thin Prep preservCyt Solution". The SS device and an aliquot of the LBC were stored at room temperature (18–24 °C) and sent to the Microbiology Department in the Hospital of Malmo for analysis.

The consultant performed a colposcopy, and according to the clinical situation, an additional cervical biopsy or a LEEP conization was performed under local anaesthesia. In order to compare the hr-HPV samples with cytology or histological biopsies, the results from the histo-pathology review were primarily used when such a sample was taken. A cytology sample was compared with the hr-HPV positivity when no histological biopsies were available. Four women had neither cytology nor histo-pathology, due to a clinical decision.

In the laboratory, the cotton tip from the self-sample was incubated with 0.5 mL saline for ten minutes at room temperature, and then the cotton tip was rinsed in the solution by pipetting. From each sample, 200 μL was then used for automatic DNA-extraction by MagnaPure (Roche) and eluted in 100 μL. Five μL was used for HPV DNA amplification by PCR with modified GP5+/6+ (MGP) primers.[15]

After amplification the Luminex-based HPV genotyping was used to identify HPV types.[16] The technique allows the detection of 38 HPV genotypes of which 18 are high-risk HPV type genotypes: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82; and five are probable high-risk type genotypes: 26, 53, 66, 67 and 69; and 19 are low-risk HPV genotypes: 6, 11, 30, 40, 42, 43, 54, 61, 62, 70, 74, 81, 83, 86, 87, 89, 90, 91 and 114.[17–19] The Luminex assay also included two broadly reactive "universal" probes and HPV DNA in samples positive only for a universal probe was typed by DNA-sequencing. Potentially hr-HPV types were classified into the group of hr-HPV types.[20]

Beta-globin real time PCR was included as a separate test of sample adequacy for PCR, where threshold (Ct) values reflect the relative amount of cells in the sample.[21] A lower Ct-value represents a relatively higher number of cells. The lowest detection level was about five diploid cells per PCR, yielding a mean threshold Ct-value of 37.2 (CV 2.8%).

The study was approved by the Regional Ethical Board, University of Lund.

3.1. Statistical analyses

The tests are based on the binomial distribution and the exact confidence intervals (CI) are given. Kappa values were calculated using standard methods.[22]

All comparisons were two-sided, and *P* values less than 0.05 were considered statistically significant. Statistical analysis was performed using SPSS (PASW) version 18.0 (SPSS Inc., Chicago, IL, USA) and OmniStat (SBU, Sweden).

4. Results

In total, 121 patients were included in the study, but 12 patients were excluded due to logistic problems during the transportation of the samples from the clinic to the laboratory. One additional patient was excluded due to an inadequate self-sampling. We included one woman who had a non-adequate Beta-globin result in the SS presenting hr-HPV DNA in the LBC-sample. The evaluation of the SS and the LBC-samples included 108 women. The mean Ct-values for the Beta-globin adequacy-test in the SS group and LBC group were 28.7 (range 21.4–39.7, CV 12.4%) and of 27.9 (range 22.3–36.9, CV 10.7%), respectively. Positive Hr-HPV DNA was found in 56 women with both tests. The SS method detected another nine (65/108 60%; 95% CI 0.50–0.69), and the LBC method eight additional positive hr-HPV individuals (64/108 59%; 95% CI 0.49–0.69) resulting in an hr-HPV positivity of 68% (95% CI 0.58–0.76) in the test cohort (Table 1a).

Table 1a
Individuals positive for high-risk HPV in SS and LBC in relation to all test persons.

	SS		Total
	No HPV or low risk HPV	High risk HPV	
LBC			
No HPV or low risk HPV	35	9	44
High risk HPV	8	56	64
Total	43	65	108