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Type-specific detection of high-risk human papillomavirus (HPV) in self-sampled cervicovaginal cells applied to FTA elute cartridge

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ABSTRACT

Background: Most procedures for self-sampling of cervical cells are based on liquid-based media for transportation and storage. An alternative is to use a solid support, such as dry filter paper media.
Objectives: To evaluate if self-sampling of cervicovaginal fluid using a cytobrush (Viba-brush; Rovers Medical Devices B.V., Oss, The Netherlands) and a solid support such as the Whatman Indicating FTA® Elute cartridge (GE Healthcare, United Kingdom) can be used for reliable typing of human papillomavirus (HPV), as compared to cervical samples obtained by a physician using a cytobrush and the indicating FTA® Elute Micro card and biopsy analysis.
Study design: A total of 50 women with a previous high-risk (HR) HPV positive test were invited to perform self-sampling using the Viba-brush and the FTA cartridge and thereafter a physician obtained a cervical sample using the cytobrush and a FTA card, together with a cervical biopsy for histology and HPV typing. Detection of HR-HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 was performed using three multiplex real-time polymerase chain reaction (PCR) assays.
Result: All samples contained sufficient amounts of genomic DNA and the self-samples yielded on average 3.5 times more DNA than those obtained by the physician. All women that were positive for HR-HPV in the biopsy sample also typed positive both by self-sampling and physician-obtained sampling. For women with a histological diagnosis of cervical intraepithelial neoplasia grades 2–3 (CIN 2–3) all three HPV samples showed 100% concordance. A higher number of women were HPV positive by self-sampling than by physician-obtained sampling or by biopsy analysis.
Conclusion: The Viba-brush and the FTA cartridge are suitable for self-sampling of vaginal cells and subsequent HR-HPV typing.

1. Background

The clinical value of HPV testing is well established[3,4,9,10,14,15] and HPV testing has better sensitivity in detecting persistent high-grade lesions than cytology in women over 35 years.[37] However, even in countries with an organized gynecological screening program not all women attend, and a recent nationwide audit in Sweden showed that 65% of all cervical carcinomas occur in non-attendees.[2] One means to overcome the problem of non-attendees is to offer women the possibility to perform self-sampling of vaginal fluid at home and send in the sample to a laboratory for HPV analysis.[38] Several international studies have shown that many of those not attending the regular screening participate in self-sampling, indicating that this is an efficient way to increase the screening coverage.[5,12,34] Self-sampling in combination with HPV testing has been shown as a suitable alternative to physician-obtained samples,[35] and the prevalence of carcinogenic HPV types has been found to be similar between vaginal and cervical specimens.[13] Most of the systems used for self-sampling include a liquid-based media for transport and storage, which requires careful handling of often hazardous solutions and complicates the transportation by regular mail. We have shown that the indicating FTA® Elute Micro card (GE Healthcare, United Kingdom) is suitable for application of cervical cells[21] and that samples collected on the FTA card are amendable to HPV testing using a real-time PCR assay.[20,30–32] Lenselink et al. recently showed that the FTA cartridge (GE Healthcare, United Kingdom), which is a

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FTA card enclosed in a plastic cassette for protection, in combination with the Viba-brush (Rovers Medical Devices B.V., Oss, The Netherlands) is a suitable collection device.[28] The FTA card has also been widely used for collecting of various other types of biological samples, and is stable for several years when stored at room temperature.[7,8,18,19,24,27,28,29,33,41]

2. Objective

To evaluate if self-sampling of cervicovaginal fluid using a Viba-brush and an indicating FTA® Elute cartridge can be used for reliable HPV typing, when compared to the analyses of samples obtained by a physician using a cytobrush and the indicating FTA® Elute Micro card and to cervical biopsy analysis.

3. Study design

3.1. Patients

This study includes 50 women that have not attended the organized screening program for ≥6 years. The women had earlier been invited to perform self-sampling test at home (Qvintip, Aprovix AB, Uppsala Sweden) for detection of HR-HPV (Hybrid Capture 2, Qiagen AB, Solna, Sweden)[38] and were all found to be HR-HPV positive at that time. At their follow-up visit (1–3 months later) to the Department of Obstretics and Gynecology, Uppsala University Hospital, they were invited to participate in the present study. The women were instructed to perform a vaginal self-sampling using the Viba-brush and apply the sample to the FTA cartridge. They were then examined by colposcopy and a sample was collected for HPV testing by a physician using a standard cytobrush and the indicating FTA elute card. In addition, a biopsy sample was taken for both histology and HPV typing.

3.2. Sample collection

All women received verbal and written instruction on how to collect a cervicovaginal sample and apply this to the FTA cartridge. Participants were instructed to pull out the inner cassette of the indicating FTA cartridge (WB 659223, GE Healthcare, United Kingdom) and place it on a clean dry surface. The brush (Rovers Viba-brush; Rover Medical Devices B.V., Oss, The Netherlands) was removed from the cover, holding it at the handgrip without touching the bristles. The brush was inserted approximately 7 cm into the vagina (similar to inserting a tampon) and gently turned twice. The brush was then removed and the sample applied to the FTA cartridge by placing the brush in the middle of the application area and rolling it one full circle across that area. The FTA cartridge was then air-dried before closing the cassette. After the self-sampling, a physician obtained a cervical smear for HPV test using a cytobrush and applying the sample to an indicating FTA elute card (Whatman, Inc., Clifton, New Jersey, art no WB120411).[21] The biopsy specimen was fixed in 10% formalin, embedded in paraffin, and 4 μm sections were stained with haematoxylin–eosin. The sections were examined in light microscope and classified into normal, cervical intraepithelial neoplasm (CIN) 1, CIN 2 and CIN 3. A 10 μm section of the cervical biopsy was also collected in a tube for subsequent detection of HR-HPV.

3.3. Retrieval of DNA from FTA cards

A 3 mm Ø Harris micro punch (GE Healthcare, United Kingdom) was used to excise 4 pieces from the FTA cartridge and the FTA cards using the BSD 600 (BSD Robotics, Queensland, Australia) semi-automatic punch robot. The 4 punches from each sample were transferred to a single well in a 96-well plate and washed by vortexing 3 × 5 s in 200 μl distilled H_2O. The water was then carefully removed with a pipette. The DNA elution was performed in 50 μl distilled H_2O at 95 °C for 30 min in a heating block (with heated lid). Three μl of the DNA extract was used as template in each real-time PCR.

3.4. DNA extraction from paraffin-embedded biopsies

The extraction of DNA from the 10 μm thin section was performed as earlier described.[25,26] Three μl DNA was used as template in each real-time PCR.

3.5. Real-time PCR assay

The typing of HPV was performed as earlier described, using a real-time PCR-based assay.[20,30] This assay detects and quantifies a human single copy gene (house keeping gene) (HMBS, Homo sapiens hydroxymethylbilane synthase; GenBank accession no. M95623.1) and the following HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59. In order to be able to determine if a sample contains sufficient amounts of material for a HPV test to be considered informative, we used a threshold of 10 copies of the nuclear single copy gene per PCR, based on the analysis of HMBS. In addition, for the HPV test itself, the sample had to contain a minimum of 10 HPV copies to be typed positive.

3.6. Statistical analysis

Kappa values were calculated using standard methods (http://www.graphpad.com/quickcalcs/Kappa2.cfm).

4. Result

4.1. Concordance between vaginal smears obtained by self-sampling and cervical smears and biopsy samples obtained by a physician

All samples types contained sufficient amounts of genomic DNA for HPV typing (i.e. ≥10 copies of the HMBS single copy gene). The sample taken by the women themselves contained on average 3.5 times more nuclear genomic DNA (mean = 7000 copies of genomic DNA) as compared to the sample taken by the physician (mean = 2000 copies of genomic DNA). Of the self-samples, 68% (34/50) were positive for HR-HPV, as compared to 56% (28/50) of the physician-obtained samples and 42% (21/50) of the biopsies. The HPV types found in all HPV positive samples are shown in Table 1. All women that were positive for HR-HPV in the biopsy specimen were also positive both by vaginal self-sampling and physician-obtained cervical cell sampling. Thus, in no case was an HR-HPV infection detected in the biopsy missed by analysis of the other samples types. The overall agreement between having a HPV infection (any of the ones included in the assay) in samples taken by the women themselves and samples obtained by the physician was estimated to 88% (kappa = 0.75, 95% confidence interval 0.56–0.94). Four women had additional multiple HPV infections solely in the self-sampling material and in six women a HPV infection was only detected by self-sampling (Table 1). All these six samples had HPV copy numbers close to the lower cut-off (data not shown) for positivity in the HPV typing assay used.[20,30] Of women that were HPV positive by both self-sampling and physician-obtained sampling, 75% (21/28) were also positive in the biopsy sample (Table 1).

4.2. Histological diagnosis and HPV typing

CIN 2–3 was found in 22% (11/50) of the women, CIN 1 in 40% (20/50) and 38% (19/50) had normal histology. Data on the histology