Direct comparison of two vaginal self-sampling devices for the detection of human papillomavirus infections

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A B S T R A C T
Background and objectives: Two devices for vaginal self-sampling of dry cell material (Evalyn Brush, Rovers Medical Devices; Qvintip, Aprovix) were compared using the Abbott RealTime High Risk HPV test.

Study design: Both self-sampling devices (change of order with every patient) including instructions for use and a questionnaire were handed to 146 patients in a colposcopy clinic prior to scheduled colposcopies with collection of cervical reference specimens by gynaecologists using a broom-like device. Matched self-collected and physician collected specimens were transferred to ThinPrep medium and tested for the presence of hr-HPV. Biopsies were taken if indicated by colposcopy.

Results: Evaluation of 136 patients with complete data (136/146; 93.2%) showed high agreement of overall hr-HPV detection rates between self-collected and clinician-collected specimens (Evalyn: 91.2% [kappa 0.822]; Qvintip: 89.0% [kappa 0.779]). Colposcopy and histological evaluation revealed 55 women without cervical intraepithelial neoplasia (CIN), 32 CIN1, 34 CIN2, 14 CIN3 and one adenocarcinoma in situ. Hr-HPV testing detected all CIN3+ cases on the clinician-taken or Evalyn self-samples (14/14) and 93% of them on the Qvintip samples (13/14). There was no significant difference regarding the specificity for CIN2+ or CIN3+ and specificity of hr-HPV testing on self- vs. clinician samples and on Evalyn vs. Qvintip. Based on signal intensities of β-globin, the observed DNA concentration with Evalyn samples (mean CN: 22.0; 95%-CI: 21.5–22.6) was found to be significantly higher compared to that of Qvintip samples (mean CN: 23.8; 95%-CI 23.2–24.4), regardless of the order of self-sampling (p < 0.0001). Most women considered self-sampling easy and comfortable. Qvintip was considered easier than the Evalyn Brush to understand (p < 0.001) and to use (p = 0.002).

Discussion: This study confirms that hr-HPV testing with a clinically validated PCR-based HPV assay is as accurate on self-samples as on clinician-samples without significant difference between both self-sampling devices.

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1. Background

High-risk human papillomavirus (hr-HPV) infection of the cervicovaginal tract is known to be the major cause of cervical cancer [1] and detection of the virus in physician-collected cervical scrapes demonstrated superior efficacy for reducing the incidence of cervical cancer compared to cervical cytology [2,3]. Various countries have announced future implementation of hr-HPV testing as a primary screening method [4–6]. However, a major factor for the success of screening is the coverage rate of the respective target population. In the EU, coverage rates vary widely between member states [7].

In Germany only 45% of the women over 20 years have yearly screening examinations as recommended. Two thirds are screened at least once in two years [8]. Among all patients diagnosed with invasive cervical cancer, 60% had not been screened for at least five years. Another 31% did not receive Pap smears in yearly intervals. Additionally, most of the so called non-responders were diagnosed with more advanced tumors (Stage T1b = clinically visible lesions and worse) whilst 54% of the cancers diagnosed during routine screening were microinvasive (Stage T1a = only diagnosed
microscopically) [9]. Similar data were reported from Sweden, The Netherlands and California [10–13].

One of the most promising concepts to improve the participation rate among non-responders is to provide self-sampling devices designed for home-collection of cervicovaginal material. HPV testing on self-collected and physician-collected cervical specimens with clinically validated PCR-based tests generally shows similar sensitivity for high-grade CIN on both sampling methods [14,15]. Numerous studies have been conducted with a diversity of individual self-sampling devices but little attention has been given to the direct comparison of different self-sampling devices.

2. Objectives

This pilot study was conducted to compare two dry vaginal self-sampling devices (Evalyn Brush, Rovers Medical Devices and Qvintip, Aprovix) in combination with the Abbott RealTime High Risk HPV test (RealTime; Abbott GmbH & Co. KG, Wiesbaden, Germany).

3. Study design

At the colposcopy clinic and the gynaecological outpatient clinic of Hannover Medical School, Germany, study participants were recruited among the patients referred for abnormal cervical screening results or general gynaecological diseases. Pregnant women and women with hysterectomy in the past were excluded. The study was approved by the institutional ethics review board prior to beginning and all participants gave written consent before enrollment.

At first, all participants were given the two sampling devices (alternating order in every patient), written and illustrated instructions as provided by the manufacturers (translated to German) and a questionnaire addressing personal and medical history and the acceptance of the self-sampling devices (ten point analogue scale; see Table 1). If there were missing answers concerning one self-sampling device on the questionnaires the answers concerning the other device were excluded from the final analysis to have equal data sets for both devices.

After completion of self-sampling procedures and questionnaires in a separate room at the clinics and without assistance by hospital staff, all women received their scheduled examination by a gynaecologist. At first, a liquid-based cervical cytology smear was taken with a broom-like device (Hologic, Marlborough, MA) and immediately suspended in 20 ml of Cytosc ThinPrep PreservCyt Solution (Hologic). This sample was also used as reference HPV sample. Then colposcopy was performed using acetic acid and taking directed biopsies and/or endocervical curettage if indicated (routine cyto- and histopathological examination). In case of unsuspicious colposcopy there were no biopsies taken. Histology was classified into low-, moderate- and severe cervical intraepithelial neoplasia (CIN 1-3) and adenocarcinoma in situ [16]. The final diagnosis for each woman was defined according to the worst histological or cytological result.

Self-samples were stored at room temperature for 8–9 days on average (range, 1–67 days) then they were thoroughly washed in 20 ml of PreservCyt Solution for at least five seconds until visible cell material was transferred to the medium. The physician-collected reference samples were at first used to prepare liquid based cytology slides with the ThinPrep 2000 system (Hologic). Then 700 μl each of the self samples and residual reference sample material were used for hr-HPV testing with the RealTime assay on the m2000 System (Abbott).

3.1. Self sampling devices

The Evalyn Brush has a broom-like collection head that can be retracted into a tube. It is about 20 cm long and has lateral wings indicating the necessary depth of insertion. After insertion, the brush has to be rotated five times, then it has to be retracted and covered by a cap and can be sent by mail. The Qvintip collection device has a solid white plastic head that is about 5 cm long and 7 mm thick. The head has multiple groves to collect the cervicovaginal cell material. The device has to be inserted as far as possible, then it is removed and the plastic head is transferred into a separate tube without touching and further manipulation. This tube can also be sent by mail, the rest of the device is disposed of.

3.2. Hr-HPV Test

The Abbott RealTime High Risk HPV test is an automated, qualitative multiplex assay based on real time polymerase chain reaction (PCR) for the detection of 14 h-HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) and simultaneous differentiation of HPV 16 and HPV 18 as described before [17]. It has been clinically validated for routine use in cervical cancer screening in different countries [17,18] and proved to be accurate on clinician samples in a referral population [19] and for hr-HPV testing of self-collected lavage samples [20].

3.3. Statistical analysis

Microsoft Excel 2010 (Microsoft Corp., Redmond, WA) and IBM SPSS Statistics 22 (IBM Corp., Armonk, NY) were used for data collection and evaluation. Cohen’s Kappa was calculated to assess the agreement between the different sampling methods and the Wilcoxon signed-rank test (two-sided p-value) was used compare the mean real-time PCR cycle number (CN) values and acceptance rates as indicated on the questionnaires [21].

4. Results

Overall, 146 women were recruited for the study. Of these, ten cases had to be excluded from the final analysis: two cases of women who initially agreed to participate but who did not perform the study procedures, three cases without reference smears and one case with only one self-sample. Four cases with invalid RealTime...
test results had to be excluded, too (per protocol analysis). All of the four reference samples had valid hr-HPV test results but there were two Evalyn samples and two Qvintip samples with insufficient cell material (β-globin above CN threshold). This resulted in one HPV 16 positive CIN 3 with an inadequate Evalyn sample and three hr-HPV negative cases with inadequate self-samples.

A total of 136 cases with complete sets of test results were included in the per protocol evaluation. Mean patient age was 36 years (range: 17–78). An overall hr-HPV positivity rate of 55% (75/136) was found in the reference sample population with mostly non-HPV 16/18 genotypes detected. Multiple infections were found in 11 specimens (8%; see Table 2).

Very high agreement of hr-HPV reactivity rates was found between self-collected samples and physician-collected reference samples (Evalyn: 91.2% [kappa 0.822; 95%-CI 0.726 – 0.918]; Qvintip: 89.0% [kappa 0.779; 95%-CI 0.674 – 0.885]; see Table 3). No significant differences between the agreement rates for self- and clinician sampling in relation to the order of self-sampling devices were used.

There were 34 CIN 2, 14 CIN 3 and one adenocarcinoma in situ among the 136 included cases. All CIN 3+ cases were detected with Evalyn, one CIN 3 was missed with Qvintip (reference and Evalyn: non 16/18 HPV positive; see Table 4). There were no significant differences between the sensitivity and specificity for the detection of CIN2+ between clinician sampling, Evalyn and Qvintip in the per protocol analysis (see Tables 5 and 6). In the intention to treat analysis,

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<tr>
<td>Results of partial hr-HPV genotyping (no. of cases with HPV 16, 18, other hr-HPV genotypes or multiple infections).</td>
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<td>N=136 included cases</td>
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<td>HPV 16</td>
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<td>Overall detection of any hr-HPV type</td>
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<td>Agreement of self- and clinician sampling.</td>
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<td>Hr-HPV positive</td>
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<td>Hr-HPV positive</td>
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5. Discussion

In many countries new guidelines are developed for cervical cancer screening [4,5,22] with HPV testing being implemented in primary screening programs. However, women refraining from screening continue to be at a high risk for developing cervical cancer [9]. Improving their participation rate is a very important way to reduce cervical cancer incidence. Self-sampling can help to reach these screening non-responders [23–25].

This is one of the first studies directly comparing the performance of two commercial self-sampling devices using a clinically validated hr-HPV DNA test. Both devices showed very similar hr-HPV detection rates and a high agreement with clinician-collected cervical samples among 136 patients. There was a detection rate of over 90% for CIN3+ and of over 83% for CIN2+ with both self-sampling devices.

We also performed an intention to treat analysis where we counted the four inadequate samples as “hr-HPV not detected”. Our study did not have the possibility to re-invite patients with invalid test results for retesting. On average, ~20% can be reached by offering self-samplers [23]. In this difficult to reach population, there is a considerable risk of non-participation after an inadequate self-sample and possible non-detection of high-grade dysplasia. However, there were no significant differences in the accuracy for CIN 2+ between the two sampling devices in both the per protocol and the intention to treat analysis.

Evaluation of the signal intensity (CN) of Internal Control (human β-globin) values reported by RealTime revealed a significantly higher overall β-globin signal with Evalyn compared to Qvintip (p <0.0001). This observation indicates that the amount of cellular material collected with Qvintip is lower than with Evalyn. It is unclear whether this is driven by the design of the devices heads or the more thorough sampling procedure (five rotations with Evalyn, one rotation with Qvintip). However, since RealTime is designed to optimize clinical performance rather than accurate quantification of pathogen load these results have to be interpreted with caution. Interestingly, the lower cellular concentration of Qvintip did not influence its clinical performance (detection of high-grade CIN) with the analytical procedures used in this study.
The acceptance of both sampling devices was high among the study population. Usage of Qvintip was considered easier than of the Evalyn Brush. Possibly the Evalyn self-sampling process is a little more complicated than using the Qvintip. Surprisingly, using the Qvintip was considered significantly easier by women between 40 and 50 years of age in comparison to women <30 years.

There have been many studies evaluating the feasibility and performance of self-sampling for hr-HPV before but only few studies compared different sampling devices with one another. Igidbashian et al. compared the acceptability of two different self-sampling methods but this was done in two separate groups of women and each woman only used one of the devices [26]. The authors report a high acceptance of self-sampling in favor of the lavage device and—in contrast to our results—a higher preference of self-sampling in comparison to clinician sampling. Recently, Bosgraaf et al. published a large study among over 30,000 screening non-responders that either received a lavage device or the Evalyn Brush [27]. The detection rates for hr-HPV and CIN in this screening population cannot be compared to our results among women referred for colposcopy. However, the evaluation of the questionnaires also showed high acceptance of both devices and only little discomfort.

Both sampling devices used in our study have been evaluated in previous studies. Evaluation of the Evalyn Brush in a similar referral setting showed an agreement with clinician-collected HPV samples of about 85% (91.2% in our study) [28]. There are several studies where Qvintip was used for self-sampling [29–34] but there was not a comparison to clinician sampling in all of them. Stenvall et al. reported a kappa value of 0.72 for hr-HPV detection using Qvintip sampling and clinician sampling (0.779 in our study) and a high acceptance of Qvintip [34].

The results of this study can only partially be transferred to a general screening situation. The women participating in this study mostly attended cervical screening in the recommended yearly intervals and the education level was rather high. Their attitude towards cervical cancer screening in general and self-sampling might differ from screening-deniers who would be the main target population for self-sampling. Additionally, the results could be different with other hr-HPV tests and can only be generalized for similar hr-HPV detection assays. Furthermore, transferring every self-sample to 20 ml of ThinPrep solution is costly, time consuming, labor intensive and comprises risk of sample mislabeling and cross-contamination and thus may not suit the needs of high-throughput routine settings. However, the pre-analytic procedure applied for self-collected samples in this study allowed to minimize the number of variables between the matched self- and clinician sampled specimens, thus minimizing potential bias. Conducting this study at a colposcopy clinic helped to motivate women to participate and to keep the dropout rate low. Still, sending all participants to a separate room for self-sampling without assistance by medical personnel should reflected the real self-sampling process quite well.

In summary, this comparative study of two dry self-sampling devices showed a very good agreement of hr-HPV positivity rates between clinician sampling and both devices and a detection rate of CIN3+ over 90% with both devices. The acceptance of self-collection was very high and most women experienced no difficulties or discomfort during the procedure. In the future the potential of alternative strategies including sending self-samplers has to be evaluated among a larger group of screening deniers to further validate their use in a general organized screening setting.

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### Conflict of interest

M. Jentschke received payment for travel expenses and lecture fees from Abbott Molecular GmbH & Co. KG, Wiesbaden, Germany. Abbott Molecular GmbH & Co. KG, Wiesbaden, Germany provided the hr-HPV test kits at reduced cost.

### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcv.2016.06.016.

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