Accuracy of dry vaginal self-sampling for detecting high-risk human papillomavirus infection in cervical cancer screening: A cross-sectional study

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HIGHLIGHTS
• HR-HPV infection was assessed by a PCR-based test on self and clinician samples.
• Vaginal self-collected dry swab is accurate to detect HR-HPV cervical infection.
• Vaginal self-collected dry swab could be used to reach unscreened women.

ABSTRACT
Objective. Cervical cancer screening coverage remains insufficient in most countries. Testing self-collected samples for high-risk human papillomavirus (HR-HPV) could be an alternative to the Pap smear, but costs, sampling methods and transport issues hamper its wide use. Our objective was to compare diagnostic accuracy of 2 vaginal self-collection methods, a dry swab (vsc-DRY) or swab in liquid medium (vsc-LIQ), for detecting HR-HPV cervical infection assessed by a cervical clinician-collected sample in liquid medium (ccc-LIQ).

Methods. Women 20 to 65 years attending a Pap smear were recruited between September, 2009 and March, 2011. Each sample (3 per woman) underwent HPV DNA testing. Samples were classified as HR-HPV+ with detection of at least one HR-HPV or probable HR-HPV type.

Results. Of 734 women included, 722 had complete HPV data. HR-HPV was detected in 20.9% of ccc-LIQ samples. Estimated sensitivity and specificity to detect HR-HPV in vsc-DRY samples were 88.7% and 92.5%, respectively, and in vsc-LIQ samples, 87.4% and 90.9%. Cytology findings were abnormal for 79 women (10.9%): among 27 samples of low-grade squamous intraepithelial lesions, 25 were HR-HPV+ in vsc-DRY, vsc-LIQ and ccc-LIQ samples. Among 6 samples of high-grade squamous intraepithelial lesions, all were HR-HPV+ in vsc-DRY samples, 1 was HR-HPV− in vsc-LIQ samples and 1 was HR-HPV− in ccc-LIQ samples.

Conclusions. Vaginal self-sampling with a dry swab is accurate to detect HR-HPV infection as compared with cervical clinician-collection and accurate as compared with cytology results. This cheap and easy-to-ship sampling method could be widely used in a cervical cancer screening program.

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Introduction

Cervical cancer is the fourth most common cancer in women worldwide, with an estimated 528,000 new cases and 266,000 deaths in 2012 [1]. Cytology-based screening has been associated with a major reduction in both the incidence of the disease and related mortality [2,3]. However, coverage of the screening, estimated at 63% in developed countries, remains insufficient [4].

Persistent infection with high-risk types of human papillomavirus (HR-HPV) is a necessary cause of invasive cervical cancer [5,6]. About 70% of invasive cervical cancers involve HPV types 16 and 18 [7]. HPV prevalence peaks in women in their early 20s but is usually transient. Therefore, HPV DNA testing has been proposed as a highly sensitive strategy for cervical cancer screening as an alternative to cytology in women older than 30 years [8–13]. HPV DNA testing of self-collected genital samples could be of interest to increase screening compliance for women who are never or seldom screened [14–18]. To our knowledge, self-sampling is not used yet in nationwide, population-based screening programs. Practical issues, especially the sampling device and transport methods, need to be evaluated in a large sample of women before the strategy can be widely implemented in large-scale clinical practice.

A great variety of self-collection devices such as swabs, brushes, tampons, pads and cervico-vaginal lavages has been tested [17,18]. Devices are usually placed in a liquid specimen transport medium at the collection point. Impractical and cost aspects of liquid transport may hinder the widespread introduction of self-collection methods in population-based programs. A dry swab would offer potential advantages in terms of collection, cost and shipment; the swab could be personally sent to unscreened women and mailed back to a laboratory.

We aimed to compare the HR-HPV diagnostic accuracy of 2 vaginal self-collection methods, a dry swab (vsc-DRY) or swab in liquid medium (vsc-LIQ), for predicting HR-HPV cervical infection assessed by a cervical clinician-collected sample in liquid medium (ccc-LIQ).

Materials and methods

This multicenter cross-sectional study compared the HR-HPV diagnostic accuracy of 2 vaginal self-collection methods and a clinician-collected sample which was also examined by cytology (i.e., Pap smear). The study was conducted between September 11, 2009 and March 9, 2011 in 4 centers: a family-planning clinic and a gynecology consultation center in the University Hospital of Tours, France; and 2 medical check-up centers in western France. The study protocol was reviewed and approved by the regional ethics committee (Tours, France) and all women provided written informed consent for inclusion in the study. The Standards for Reporting of Diagnostic Accuracy (STARD) statement was used for reporting the study [19]. The study registration number was NCT01014026 (clinicaltrials.gov).

Participants

Women who were due for a routine screening Pap smear were eligible if they were 20 to 65 years old, self-reported not a virgin, not pregnant, not vaccinated against HPV, not menstruating, had not had a Pap smear for at least 2 years and had no prior hysterectomy.

Study design

Three genital samples were taken from each subject on the same day during a medical consultation: a vsc-DRY swab collection, a vsc-LIQ swab collection placed in a vial containing a liquid transport medium, and a ccc-LIQ brush collection placed in a vial containing a liquid transport medium.

Sample collection

Nylon flocked swabs were selected for their good cell-sample collection–elution capabilities, low price and design, considered more “female-friendly” than other self-collection devices (brush, lavage). Women were given a self-collection kit that included 1) a leaflet designed in collaboration with a medical illustrator with written and cartoon instructions detailing how to perform the 2 vaginal self-collections (eFigure 1); 2) for vsc-DRY samples, an envelope containing a nylon flocked swab in a non-breakable sterile tube (53080C, Copan, Brescia, Italy); and 3) for vsc-LIQ samples, an envelope containing a nylon flocked swab with a molded breakpoint on the swab shaft that was enclosed in a sterile peel pouch (509SCS01, Copan, Brescia, Italy) and a 12 × 80-mm screw cap tube containing 2 mL transport and preservation liquid medium (610C, Cymol, Copan, Brescia, Italy). The 2 envelopes were numbered to indicate the sequence for the self-collections. The sequence for the 2 self-samples was a priori determined by computer-generated randomization. The women performed the self-collections in the clinician’s office or near the clinician’s office (in toilets or in a changing room) before the clinical examination and collection.

Cervical clinician collection was performed after self-collection as we assumed that speculum examination could reduce vaginal self-collection performance (friction of the speculum on the vaginal wall); conversely, we assumed that self-collections were less likely to reduce the quality of cervical sampling because they should be performed in the lower part of the vagina, not in the cervix.

The clinician performed a pelvic and speculum examination during which a cervical specimen was collected (ccc-LIQ) as for liquid-based cytology. Ectocervical and endocervical cells were collected with use of a Cervexbrush (Rovers Medical Devices B.V., Oss, The Netherlands) and were resuspended in a specimen transport liquid medium (Thinprep Paptest, Presercyt solution, Hologic, Bedford, MA, USA). The vsc-DRY and vsc-LIQ samples were placed in sealed envelopes that were anonymously labeled with a unique sample code and shipped separately at ambient temperature by the clinician’s staff to the centralized virology laboratory for HPV DNA testing (University Hospital of Tours, France). The ccc-LIQ samples were shipped at ambient temperature to the cytology laboratory (Institut inter-Régional pour la Santé, Tours, France), where an aliquot was taken for liquid-based cytology analysis; the remaining sample was then shipped to the virology laboratory for HPV DNA testing.

Acceptability of sampling

To ensure that women had access to the same information they would have with home self-collection sampling, the clinician could not help them carry out the 2 self-collections or answer questions related to the protocol. After self-collection, women were asked to report whether they had difficulties and whether the sampling procedure was painful.

Blinding during sample analysis

Each sample was identified with a unique number, with no possibility for biologists to associate the 3 samples with a subject during HPV DNA analysis. Moreover, virology and cytology laboratory testing were performed without knowledge of the subject’s other test results.

Laboratory testing

Cytology — Pap smear

Cytology slides were produced for all ccc-LIQ samples according to the manufacturer’s specifications (Hologic, Bedford, MA, USA) and were evaluated in the cytology laboratory by a single well-trained cytopathologist using the 2001 Bethesda system [20]. Cytology findings were labeled as unsatisfactory, normal, atypical squamous cells of undetermined...
Between September 11, 2009 and March 9, 2011, 734 women were recruited. The study was stopped prematurely because the 133 required women with positive HR-HPV DNA test results for ccc-LIQ samples had been included. We excluded 12 women: 2 ccc-LIQ and 3 vsc-DRY samples were unavailable, and 1 ccc-LIQ and 6 vsc-DRY samples were uninterpretable for HPV DNA testing. Therefore, the analysis was restricted to 722 women (98.4%) with complete HPV data (i.e., 3 HPV DNA test results) (Fig. 1). Most women (74.2%) were 30 to 65 years old.

HPV prevalence

HR-HPV prevalence was lower in ccc-LIQ samples (151/722, 20.9%) than in vsc-DRY samples (177/722, 24.5%, p = 0.0008) and in vsc-LIQ samples (184/722, 25.5%, p > 0.0001), (Table 1). HPV prevalence did not differ by whether the dry or liquid self-collection was performed first (data not shown).

Typing failure

Among 2166 samples, HPV was untypable in 100 samples (corresponding to 56 women): we could successfully sequence 10/28 (all LR-HPV), 17/38 (1 HR-HPV and 16 LR-HPV) and 14/34 (2 HR-HPV and 12 LR-HPV) ccc-LIQ, vsc-DRY and vsc-LIQ samples, respectively. Sequencing results were not considered for agreement analysis.

Diagnostic accuracy and agreement

The estimated sensitivity and specificity to detect HR-HPV in vsc-DRY samples were 88.7% (95% CI [82.6; 93.3]) and 92.5% (95% CI [90.0; 94.5]) respectively, and in vsc-LIQ samples 87.4% (95% CI [81.0; 92.3]) and 90.9% (95% CI [88.2; 93.1]). Kappa values for the HPV DNA test results on the paired samples were almost perfect between the 2 self-collected samples and substantial between the clinician-and-self-collected samples according to the Landis and Koch classification [24] (Table 2).

For vsc-DRY and vsc-LIQ samples, the estimated sensitivity to detect HPV 16 and/or 18 was both 75.0% (95% CI [58.8; 87.3]) and associated Kappa values were substantial. Estimated specificity to detect HPV 16 and/or 18 was 98.2% (95% CI [96.9; 99.1]) for vsc-DRY and 97.5% (95% CI [96.0; 98.5]) for vsc-LIQ (Table 2).

Comparison of cytology and HPV DNA test results

In all, 79 women (10.9%) had abnormal cytology results; HR-HPV was detected in 64.6%, 65.8%, and 67.1% of the ccc-LIQ, vsc-DRY and vsc-LIQ samples, respectively. Among 46 ASC-US, 21 were HR-HPV+ in ccc-LIQ and vsc-DRY samples and 23 were HR-HPV+ in vsc-LIQ samples. Among 27 LSIL, 25 were HR-HPV+ in vsc-DRY, vsc-LIQ and ccc-LIQ samples. Among 6 HSIL, all were HR-HPV+ in vsc-DRY samples and 1 was HR-HPV− in vsc-LIQ samples and 1 was HR-HPV− in ccc-LIQ samples (Table 3).

Among the 634 normal cytology results, HR-HPV was detected in 15.6%, 19.6% and 20.5% of the ccc-LIQ, vsc-DRY and vsc-LIQ samples, respectively. The 9 remaining samples had unsatisfactory cytology results (Table 3).

The 17 women with negative vsc-DRY but positive ccc-LIQ results had no abnormal cytology findings; one HSIL case was found among 24 cases of HR-HPV− in ccc-LIQ samples and HR-HPV+ in vsc-DRY samples; one case each of HSIL and ASC-US were found among 19 cases of HR-HPV+ in ccc-LIQ samples and HR-HPV− cases in vsc-LIQ samples; one HSIL case and 3 ASC-US cases were found among 52 cases of HR-HPV+ in ccc-LIQ samples and HR-HPV− cases in vsc-LIQ samples (Table 3).

Difficulties or discomfort

In all, 13 women (1.8%) asked for the clinician’s help during the self-sampling procedure. A total of 104 women (14.4%) reported experiencing difficulties performing the vaginal self-collection. They had trouble
estimating the required depth for the self-collection or where and how to introduce the swab; 64 women (8.9%) reported pain or discomfort during the self-collection procedure.

Discussion

In a large sample of 722 women, we demonstrated that vaginal self-collection is an accurate method to detect cervical HR-HPV infection and cytological abnormalities as compared with a cervical clinician-collection method. Moreover, these results apply to both liquid and dry and cytological abnormalities as compared with a cervical clinician-collection method. Therefore, this technique can be easily shipped by regular mail, and the method is accurate for HR-HPV detection in a population-based program. However, we aimed to assess a transport liquid medium; reference standard was the HPV test performed on the cc-LIQ.

Table 1

<table>
<thead>
<tr>
<th>HPV oncogenic group</th>
<th>Sampling method</th>
<th>HPV prevalence, N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age (years)</td>
<td>≤30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(N=186)</td>
</tr>
<tr>
<td>Any HPV</td>
<td>ccc-LIQ</td>
<td>81(43.6%)</td>
</tr>
<tr>
<td></td>
<td>vsc-DRY</td>
<td>86(46.6%)</td>
</tr>
<tr>
<td></td>
<td>vsc-LIQ</td>
<td>87(46.8%)</td>
</tr>
<tr>
<td>HR-HPV</td>
<td>ccc-LIQ</td>
<td>65(35.0%)</td>
</tr>
<tr>
<td></td>
<td>vsc-DRY</td>
<td>70(37.6%)</td>
</tr>
<tr>
<td></td>
<td>vsc-LIQ</td>
<td>70(37.6%)</td>
</tr>
<tr>
<td>HPV 16/18</td>
<td>ccc-LIQ</td>
<td>16(8.6%)</td>
</tr>
<tr>
<td></td>
<td>vsc-DRY</td>
<td>16(8.6%)</td>
</tr>
<tr>
<td></td>
<td>vsc-LIQ</td>
<td>18(9.7%)</td>
</tr>
<tr>
<td>LR-HPV and additional types</td>
<td>vsc-DRY</td>
<td>28(15.1%)</td>
</tr>
<tr>
<td></td>
<td>vsc-LIQ</td>
<td>33(17.7%)</td>
</tr>
</tbody>
</table>

Because dry samples would be easier and less costly to send via regular mail than samples in vials containing a liquid medium, vaginal dry self-collection (brushes, Dacron or cotton swabs) was assessed in 4 studies, each with fewer than 140 women, with fairly good agreement with clinician-collected findings [26–29]. Four other studies, involving filter-paper cards, which are more expensive than swabs or brushes, also showed high agreement with limited samples [30–33].

One limitation of the study is that one quarter of included women was younger than 30 years, and they would not be targeted in a HPV population-based screening program. However, we aimed to assess a device and a sampling method rather than HPV prevalence. Otherwise, to our knowledge, this study is the first to compare, with a large sample, 3 matched biological results: HPV detection by dry and liquid vaginal self-collection, and HPV detection by cervical clinician collection. These 3 biological results were also compared to cytology results.

In conclusion, self-collection of vaginal samples with use of a dry nylon flocked swab is “female-friendly” and cheap, products could easily be shipped by regular mail, and the method is accurate for HR-HPV detection in a population-based program.

Conflict of interest statement

All authors have signed the “Conflict of Interest Policy Form”. Dr Haguenoer reported receiving for his institution grant support from the French National Cancer Institute and French League Against Cancer; receiving support from Innogenetics and Sanofi Pasteur MSD for travel expenses to HPV congress; participating without compensation in meetings on cervical cancer prevention organized by Sanofi Pasteur MSD and GlaxoSmithKline. Dr Gaudy-Graffen reported receiving support from Innogenetics for travel expenses to HPV congress. Dr Viguier reported being a member of the French National Cancer Institute. Dr Marret reported being invited to international congress on HPV and participating without compensation in meetings on cervical cancer prevention by Sanofi Pasteur MSD and GlaxoSmithKline. The remaining coauthors report no financial disclosures.

We did not use any writing assistance but used English language editing services.

Acknowledgments

Study investigators

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Table 2

Diagnostic accuracy and agreement between self- and clinician-collected samples in detecting HPV.

<table>
<thead>
<tr>
<th>HPV DNA test results</th>
<th>Sensitivitya [95% CI]</th>
<th>Specificitya [95% CI]</th>
<th>Kappa [95% CI]</th>
<th>Agreement [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection of HR-HPV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vsc-DRY HR-HPV+</td>
<td>134</td>
<td>43</td>
<td>177</td>
<td>88.7%</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>528</td>
<td>545</td>
<td>[82.6–93.3]</td>
</tr>
<tr>
<td>Detection of HPV16 and 18</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vsc-DRY HPV16/18+</td>
<td>30</td>
<td>12</td>
<td>42</td>
<td>75.0%</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>670</td>
<td>680</td>
<td>[58.8–87.3]</td>
</tr>
</tbody>
</table>

Abbreviations: HPV, human papillomavirus; HR-HPV+, positive for high-risk HPV and/or probable high-risk HPV; HR-HPV−, negative for high-risk HPV and probable high-risk HPV; ccc-LIQ, cervical clinician-collected sample in transport liquid; vsc-DRY, vaginal self-collected dry swab; vsc-LIQ, vaginal self-collected swab in a transport liquid medium. 95% CI, 95% confidence interval.

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The decision to publish was made by the study investigators.

Author contributions

Haguenoer had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Haguenoer, Giraud, Marret, Goudeau.

Acquisition of data: Haguenoer, Giraud, Gaudy-Graff, de Pinieux, Dubois, Trignol-Viguière, Marret, Goudeau.

Analysis and interpretation of data: Haguenoer, Giraud, Gaudy-Graff, Goudeau.

Critical revision of the manuscript for important intellectual content: Haguenoer, Giraud, Gaudy-Graff, de Pinieux, Dubois, Trignol-Viguière, Viguier, Marret, Goudeau.

Statistical analysis: Haguenoer, Giraud, de Pinieux, Dubois, Trignol-Viguière, Viguier, Marret, Goudeau.

Obtained funding: Haguenoer, Giraud, Viguier, Marret, Goudeau.

Administrative, technical, or material support: Haguenoer, Giraud, Gaudy-Graff, de Pinieux, Dubois, Trignol-Viguière, Viguier, Marret, Goudeau.

Virology analysis: Gaudy-Graff, Dubois, Goudeau.

Cytology analysis: de Pinieux.

Study supervision: Haguenoer, Giraud, Marret, Goudeau.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ygyno.2014.05.026.

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Table 3

Cytology results by high-risk HPV detection in self- and clinician-collected samples.

<table>
<thead>
<tr>
<th>HPV DNA test results</th>
<th>Number of women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pap smear resultsa</td>
<td>Unsatisfactory (N = 9)</td>
</tr>
<tr>
<td>Detection of HR-HPV</td>
<td>ccc-LIQ</td>
</tr>
<tr>
<td>Detection of HR-HPV</td>
<td>vsc-LIQ</td>
</tr>
<tr>
<td>Detection of HR-HPV</td>
<td>vsc-DRY</td>
</tr>
<tr>
<td>Detection of HR-HPV</td>
<td>ccc-LIQ</td>
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<td>Detection of HR-HPV</td>
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<tr>
<td>Detection of HR-HPV</td>
<td>vsc-LIQ</td>
</tr>
<tr>
<td>Detection of HR-HPV</td>
<td>vsc-DRY</td>
</tr>
</tbody>
</table>

Abbreviations: HPV, human papillomavirus; HR-HPV+, positive for high-risk HPV and/or probable high-risk HPV; HR-HPV−, negative for high-risk HPV and probable high-risk HPV; ccc-LIQ, cervical clinician-collected sample in transport liquid; vsc-DRY, vaginal self-collected dry swab; vsc-LIQ, vaginal self-collected swab in a transport liquid medium. 95% CI, 95% confidence interval; ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesions; HSIL, high-grade squamous intraepithelial lesions.

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