VALHUDES: A protocol for validation of human papillomavirus assays and collection devices for HPV testing on self-samples and urine samples

VALHUDES: a protocol for VALidation of HUman papillomavirus assays and collection DEvices for HPV testing on Self-samples and urine samples

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ABSTRACT

Back ground

Systematic reviews have concluded that hrHPV DNA testing using target-amplification tests is as accurate on vaginal self-samples as on clinician-taken specimens for the detection of cervical precancer. However, insufficient evidence is available for specific HPV assay/self-sample device combinations.

Objectives

The VALHUDES protocol is designed as a diagnostic test accuracy study that aims to compare the clinical sensitivity and specificity of particular hrHPV assay(s) on vaginal self-samples and first-void-urine, collected in agreement with standardized protocols, with hrHPV testing on matched clinician-taken samples.

Study design

Five hundred enrolled women referred to a colposcopy clinic are invited to collect a first-void urine sample and one or more vaginal self-samples with particular devices before collection of a cervical sample by a clinician. Sample sets are subsequently analysed in a laboratory accredited for HPV testing. Disease verification for all enrolled patients is provided by colposcopy combined with histological assessment of biopsies.

Results

A first VALHUDES study has started in Belgium in December 2017 with enrolment from four colposcopy centres. The following assays are foreseen to be evaluated: RealTi*m*e High Risk

HPV assay (Abbott), cobas-4800 and -6800 (Roche), Onclarity (BD), Xpert HPV (Cepheid) and Anyplex II HPV HR (Seegene).

Conclusion
Given empirical evidence that the relative accuracy of HPV-testing on self- vs clinician-samples is robust across clinical settings, the VALHUDES protocol offers a framework for validation of HPV assay/self-sample device combinations that can be translated to a primary screening setting.

BACKGROUND

Offering devices which allow women to take a self-sample may increase uptake for cervical cancer screening among particularly those who do not participate in the regular programme. Several studies have shown that mailing a self-sampling kit to the women's home address generates a greater response compared to mailing of reminder letters recommending collection of a cervical sample by a health professional[1,2]. However, the magnitude of this response gain is very heterogeneous across studies, which suggests that the impact of self-sampling depends on local conditions, context and the design of the screening programme[2]. Implementation of strategies using self-sampling without knowledge of possible effects and influencing factors may result in small gains in population coverage but substantial costs for the health budget[3,4]. The opt-in approach where non-attending women are invited to order a self-sampling device is designed to reduce waste of self-sampling devices and thereby reduce cost of operations[5,6]. However, systematic reviews of randomised trials indicate that opt-in strategies generate lower participation rates compared with mail-to-all strategies[2,5,7]. Nevertheless, several demonstration studies using opt-in strategies were successful as well in mobilising women who did not respond to an invitation to have a Pap smear taken by a clinician overall generating a considerable prevention effect[6,8]. Whether a mail-to-all or opt-in approach is chosen, finding validated and safe procedures using devices which are affordable, easy to mail, acceptable, user-friendly, technically robust and compatible with used DNA extraction and detection methods are of paramount importance[9,10][11].

An increasing number of human papillomavirus (HPV) tests are now considered as acceptable for use in primary cervical cancer screening on clinician-collected cervical cell samples[12-14]. A recent meta-analysis demonstrated that high-risk (hr) HPV assays, based on a principle of signal-amplification were significantly less sensitive and specific for underlying high-grade cervical intra-epithelial neoplasia (CIN of grade 2 or worse [CIN2+]) on self-samples than on clinician-taken samples. However, testing withPCR-based hrHPV assays, already validated on clinician-taken samples[14], showed similar accuracy on vaginal self-samples compared to cervical samples taken by a health professional[15,16]. Sub-group meta-analysis did not reveal important self-sampling device effects, with the caveat that most studies directly compared the performance of (only) one particular self-sampling device with clinician-collected specimens, and as such no strong conclusions could be drawn regarding the impact or lack of impact of the choice of the self-samplers on the test accuracy.

Among methods for self-sampling, collection of urine samples is to be considered as well. Urine sampling is in some context more culturally and religiously acceptable than cervico-vaginal based self-sampling[17]. A recent review assessed the analytical sensitivity and

specificity of hrHPV testing in urine using presence of hrHPV in a clinician-collected cervical sample as endpoint[18]. The pooled sensitivity and specificity estimates were 77% (95% CI: 68-84%) and 88% (95% CI: 58-97%), respectively. Findings were however, very variable (high inter-study heterogeneity, and included a mixture of clinical settings [screening, follow-up, age ranges, random or first-void collection, procedures for sample handling, etc.]. In addition, very few data on clinical accuracy of hrHPV testing on urine specimens are available. The few studies which have assessed the clinical sensitivity for CIN2+ have shown lower clinical sensitivity of hrHPV testing in urine than in cervical samples[19,20]. In these studies, urine sample collection may have been suboptimal for the detection of HPV given recent evidence focusing on the capture of a first-void specimen as well as addition of preservation buffers which together has the potential to significantly enhance the sensitivity of hrHPV DNA detection in urine[21-25].

OBJECTIVES

The VALHUDES project aims to provide high quality comparative data on clinical performance of HPV testing on self-collected samples. The current protocol compares the clinical accuracy of hrHPV testing with defined, clinically validated PCR-based hrHPV assays on vaginal self-samples collected with various devices and on first-void urine, collected under standardised conditions, with hrHPV testing on matched clinician-taken samples.

STUDY DESIGN

Study questions

The main objective of VALHUDES is to assess whether HPV testing on vaginal self-samples or a first-void urine specimen, using a particular self-sampler, is as accurate as HPV testing on a cervical sample taken by a clinician to detect cervical precancer. Secondary objectives include the assessment of the absolute accuracy of each HPV test applied according to each sampling device, the proportion of adequate specimens as determined by amplification of an internal control (an ubiquitous human gene), the test positivity rate, relative and absolute predictive values. CIN2+ and CIN3 or cervical carcinoma *in situ* or worse (CIN3+) are the disease outcomes.

Study design

VALHUDES is designed as a diagnostic test accuracy study following the STARD guidelines[26], where all subjects are tested independently with an index and a comparator test and subsequently are submitted to a reference or gold standard. Collections of specimens used for testing and verification of disease status occur quasi simultaneously. The index tests are an HPV assay applied on a vaginal self-sample and on a first-void urine specimen, whereas the comparator test is the same HPV assay applied on a cervical liquid-based cytology sample collected by a trained clinician. Disease verification entails colposcopy applied to all women followed by colposcopy-directed biopsy. In case of multiple biopsy episodes, the worst histological outcome will be recorded. Negative colposcopy is accepted as providing sufficient ascertainment for absence of cervical precancer.

Study population and clinical setting

Women attending a colposcopy clinic referred due to previous cytological abnormalities, HPV infection or because of suspicious symptoms will be enrolled after obtaining informed consent. From a previous meta-analysis, it was concluded that the relative sensitivity and specificity of HPV testing on self-taken compared to clinician-collected samples were similar in screening and follow-up settings. For reasons of statistical power, it is more convenient to conduct a diagnostic trial in a colposcopy setting, where all women are referred for diagnostic evaluation

and where the application of the gold standard is required for clinical reasons (rather than a study-driven intervention). This minimises the requirement for additional interventions beyond standard of care and, additionally, biases induced by partial diagnostic verification are avoided. Exclusion criteria are: women younger than 25 or older than 64, hysterectomised women, women with known pregnancy at consultation and non-consenting women and inability to understand the patient materials and informed consent form.

Study size

The sample size was computed using a method for demonstrating non-inferiority in studies with matched design[27]. Expected probabilities (sensitivity or specificity of the standard test = hrHPV with validated PCR in follow-up settings) were derived from a recent meta-analysis[15]. Expected discordance (hrHPV positivity/negativity) of hrHPV PCR on self-samples taken with the new device vs Evalyn Brush (Rovers Medical Devices B.V., Oss, The Netherlands) were derived from a study conducted in Hannover[28]. We accepted alpha=0.05, beta=0.20, lower confidence interval for relative sensitivity and specificity (index/comparator)=0.90 and 0.95, respectively, which yielded a need to enrol 118 CIN2+ cases and 183 ≤ CIN1 cases. The yearly number of referred patients, the proportion accepting enrolment in the study, the biopsy rate (proportion biopsied among women undergoing colposcopy), the expected prevalence of CIN2+ and ≤CIN1 have to be taken into account, to determine the required sample size in a specific colposcopy clinic. The non-inferiority in sensitivity for CIN2+ is expected to determine the study size, rather than the specificity. 118 CIN2 cases are expected to be retrievable among 353 participating colposcopy patients with biopsy. As a template we can put forward 500 included colposcopy patients, absorbing most assumptions and local statistics.

Evaluated tests and devices

In the first VALHUDES study, conducted in Belgium, the following collection procedures are foreseen: a) first-void urine collection using the Colli-Pee device (Novosanis NV, Wijnegem, Belgium) one day before the colposcopy visit; b) vaginal self-sampling with Multi-Collect Swab (Abbott Molecular, Inc., Des Plaines, IL, USA), c) vaginal self-sampling of half of the study population with Evalyn Brush and the other half with Qvintip (Aprovix AB, Uppsala, Sweden) at the colposcopy centre and d) the collection of a cervical liquid-based cytology sample by a gynaecologist using the Cervex-Brush (Rovers Medical Devices) as recommended in the European guidelines for preparation of cervical liquid-based cytology samples[29]. After collecting cervical epithelial cells, the Cervex-Brush will be pressed vigorously against the bottom of a vial containing 20 mL PreservCyt (Hologic Inc., Bedford, MA, USA) to remove all the cellular material.

The dry self-sampling devices will be transferred after arrival in the laboratory into a storage medium: the Multi-Collect Swab into Abbott Cervi-Collect buffer and the Evalyn Brush or Qvintip into PreservCyt. Colli-Pee is a non-invasive self-sampling device for collection of the first 20 mL of urine (first-void) from women or men. The collector is prefilled with a solution of Urine Conservation Medium (UCM) buffer appropriate for molecular testing for detection of infectious agents such as HPV[22]. Detailed user instructions for the Colli-Pee, Multi-Collect Swab, Evalyn-Brush and Qvintip can be found in the Belgian VALHUDES protocol in the Supplementary Appendix.

The first assays that will be evaluated are the Abbott RealTi*m*e High Risk HPV assay(Abbott GmbH & Co. KG, Wiesbaden, Germany)[30-32], and the cobas 4800 and 6800 (Roche Molecular System, Pleasanton, CF, USA)[33,34] which identify DNA of the L1 gene of

HPV16 and HPV18 separately and the pool of 12 other hrHPV types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68). In addition, the RIATOL qPCR HPV genotyping assay[35] will be evaluated, which identifies DNA of the E6/E7 genes of the same 14 hrHPV types, separately, as well as HPV types 6, 11 and 53 and the L1 gene of HPV67. All these assays use the amplification of the beta-globin gene as a sample validity or process control.

Aliquotting and further testing of archived VALHUDES specimens
Residual material of all VALHUDES specimen will be aliquoted and stored, as explained in the Supplementary Appendix, following procedures and agreements with laboratories and manufactures as applied previously in the VALGENT framework, used for validation of HPV genotyping tests applied on clinician-taken samples[36].

Attitudes and acceptance by women
The attitudes and preferences of eligible women can be evaluated by questionnaires (see Supplementary Appendix). Response rates (proportion of approached women that accept to participate in the study) will be used to monitor the enrolment statistics.

Future VALHUDES studies
Other assays expected to be evaluated through the Belgian VALHUDES framework are: BD Onclarity HPV Assay (BD Diagnostics, Sparks, MD, USA)[37], Xpert HPV Assay (Cepheid, Sunnyvale, USA)[38], and Anyplex II HPV HR (Seegene, Seoul, Korea)[39,40]. Additional assays may be included as long as residual aliquots are available.

More VALHUDES studies will be conducted in other countries and will include other self-sample device/storage medium/HPV assay combinations. This will generate a uniquely rich database that will allow further meta-analytical pooling and form a framework for universal validation and comparison of HPV assays on self-samples (vaginal or urine).

RESULTS
The Belgian VALHUDES has started in December 2017 and will enrol women from four colposcopy centres (University Hospitals of Antwerp, Brussels and Ghent and Heilig-Hart Hospital of Tienen). The protocol was approved by the Ethical Boards of the respective study centres. Residual cervical and vaginal material will be archived in the AML laboratory whereas residual first-void urine samples will be archived in the Centre for the Evaluation of Vaccination (CEV) at the University of Antwerp. Colposcopy and biopsy results will be obtained from the four colposcopy centres and there affiliated histopathology laboratories. The Unit of Cancer Epidemiology of the Belgian Cancer Centre (Sciensano, Brussels) will coordinate the study and perform statistical analyses. An interim analysis after assessment of samples from 100 women and a final analysis after assessment of all 500 samples are foreseen in mid-2018 and early 2019, respectively.

DISCUSSION
The VALHUDES protocol aims to compare the clinical accuracy of HPV testing on vaginal self-samples and first-void urine specimens collected under standardised conditions, with HPV testing on matched clinician-taken samples.

Self-sampling is becoming increasingly important and applicable to supplement organised cervical screening program or as screening modality in areas without organised cervical screening. Consequently, diagnostic test accuracy studies should preferentially be conducted in the setting where it is intended to be used. However, empirical evidence indicates that the

relative accuracy of self- vs clinician-taken specimens is robust and translatable from referral to primary screening settings[15]. This finding offers the methodological basis for VALHUDES, which generates two major advantages: efficiency and avoidance of partial verification bias. Partial verification bias typically arises when unequal proportions of test-positive and test-negative subjects are submitted for verification of disease status with a valid reference standard[42,43]. More CIN2+ patients are found in a referral population such as a colposcopy centre than in a screening population, which increases the power to address sensitivity hypothesis. Moreover, in a colposcopy centre, it is straightforward to apply the verification of disease status to all enrolled patients, and consequently, the problem of verification is avoided. Partial verification and methods to control its impact by referring a random fraction of screen-negative subjects for disease verification by colposcopy/histology, imply major methodological and statistical challenges in screening studies[42,44-47].

Reflex cytology combined or not with genotyping for HPV16/18 are recommended in several guidelines for management of hrHPV-positive women[48]. Whereas HPV16/18 genotyping can be easily applied on self-samples (vaginal and first-void urine), cytology on self-samples is not recommended due to the substantially lower sensitivity compared to cytology on a clinician-taken specimen[49-51] and the higher rates of suboptimal sample adequacy[49]. Consequently, molecular rather than morphological triage approaches obviate the need for an additional clinic visit for women who are hrHPV-positive on their self-sample which is likely to compromise the efficiency of a strategy offering self-sampling kits to unscreened or under-screened women. Identifying molecular methods applicable to self-samples that serve as a triage of hrHPV-positive women is therefore considered a priority for future research. VALHUDES studies could be easily extended to incorporate a triage component, where aliquots of hrHPV-positive women are tested for promising markers such as methylation of certain viral or human genes or micro-RNA profiles which are associated with progressive HPV infection and neoplastic transformation[23,52,53].

Urine collection might be more socially and/or religiously acceptable or simply more comfortable for some women who are reluctant to perform vaginal self-sampling. Offering an appropriate kit for collection of a vaginal self-sample or a first-void urine sample might be particularly suited in settings when directly provided to patients, e.g. during a visit to a primary care service, who are identified as not screened. A small trial conducted in a group of general practices in Brussels reported a 78% response rate when a vaginal self-sampling kit was offered directly to women not screened since three year or more[54]. Large-scale trials should be set up to confirm these promising results. Offering a self-sampling instrument to eligible patients in contact with primary care services might simplify the logistics of mailing devices[55].

This VALHUDES study can easily be reproduced in other countries and settings and may assist in facilitating the generation of more comparative data sets for different combinations of defined self-sample device and HPV assay combinations. We invite researchers to conduct other VALHUDES-like studies which could then be pooled in individual-patient-data meta-analyses. The demonstration of similar sensitivity and specificity for CIN2+ of a given HPV assay on a (vaginal or first-void urine) self-sample taken with a particular device compared to a sample taken by a clinician could be a plausible validation principle under the condition that the HPV assay fulfils international requirements for use on clinician-taken cervical specimens[13].

Acknowledgements/conflict of interest:

The VALHUDES project is a researcher-induced study, where manufacturers of HPV assays and devices for vaginal self-collection of urine-collection can participate. For the first phase of VALHUDES support was received from Abbott Molecular, Inc. (Des Plaines, IL, USA), Novosanis NV (Wijnegem, Belgium), University of Antwerp (Antwerp, Belgium), Rovers Medical Devices B.V. (Oss, The Netherlands) and Aprovix AB (Uppsala, Sweden).

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A. Vorsters is co-founder and board member of Novosanis NV a spin-off of the University of Antwerp.
I. Benoy, D. Vanden Broeck and J. Bogers are employed by AML, a commercial lab performing cervical cytology and HPV testing.

Registration:
The study was registered in ClinicalTrials.gov (identifier: NCT03064087).

Ethics approval:
Informed consent will be obtained from all individual participants enrolled in this study. The study was approved by the central Ethics Committee of University Hospital of Antwerp/University of Antwerp (B300201733869) and the local Ethics Committees of University Hospital of Brussels, University Hospital of Ghent and Hospital of Tienen.

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