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# EUROArray HPV test accuracy for cervical precancer in self- vs. clinician-collected samples using the VALHUDES protocol

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## HIGHLIGHTS

- Clinical accuracy of EUROArray HPV is similar on vaginal and first-void urine self-samples vs. clinician-collected samples.
- Clinical accuracy on Evalyn Brush samples is superior than on Qvintip, although not significant.
- Moderate to excellent inter-sample agreement was observed for overall high-risk and type-specific HPV.

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## ABSTRACT

*Objective.* Human papillomavirus (HPV) testing on self-collected urine and vaginal samples has shown great potential for cervical cancer screening by offering a non-invasive and approachable alternative to un(*der*) screened populations. Although many HPV tests were validated on cervical samples, data regarding clinical performance on self-samples is warranted prior to its clinical use.

*Methods.* The VALHUDES framework was designed to evaluate the accuracy of HPV tests on self-samples. As such, five colposcopy clinics enrolled patients with aberrant cervical results, asking them to collect a first-void urine (Colli-Pee) and a vaginal self-sample (Evalyn Brush or Qvintip). Additionally, a clinician-collected cervical sample was collected as comparator. 0.4 mL of all samples was used for DNA extraction with Abbott *m2000,* eluting in 50 μL. To detect high-risk HPV, PCR was conducted on 5 μL DNA extract from all samples with the EUROArray HPV test. Disease outcome was determined by colposcopy with or without biopsy. Relative accuracy was estimated for each self-sample type compared to the clinician-collected sample.

*Results.* Data was available from 491 and 494 matched samples for vaginal and first-void urine self-samples, respectively. Relative sensitivity for CIN2+ was 0.96 (95 % CI: 0.91–1.02) for vaginal self-samples and 0.96 (95 % CI: 0.90–1.05) for first-void urine. The specificity for <CIN2 was also not significantly lower on the self-samples compared to clinician-taken samples (relative specificity: 0.98 [95 % CI: 0.91–1.07] for vaginal self-samples and 0.94 [95 % CI: 0.85–1.04] for first-void urine).

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*Conclusions.* The accuracy of EUROArray HPV is similar on vaginal self-samples and first-void urine compared to clinician-collected cervical samples.



## 1. Introduction

Over the past years, self-collected samples have emerged as promising advancement in expanding access to cervical cancer screening programs, enabling individuals to participate by collecting a sample at home [1–4]. This approach aligns directly with the World Health Organization's strategy to accelerate cervical cancer elimination by reducing the incidence to below 4 per 100,000 by the end of the century [5]. The suitability of self-samples for screening is justified by their capacity to collect cervical secretions containing viral and human DNA [6,7]. Vaginal self-samples (VSS) capture cervical secretions within the vagina, whereas urine captures genital secretions built up around the external female genitalia which are flushed away with the first part of the urine stream, i.e., the first-void urine (FVU). This also explains why the initial part of the urine stream contains more cervical material than the subsequent flow [6,8,9]. Both sample types have shown to capture human papillomavirus (HPV). Indeed, HPV testing is a superior screening method due to the virus's central role in the carcinogenic process [10]. HPV testing on clinician-collected cervical samples (CCS) has therefore also consistently proven more effective than conventional cytological methods in preventing cervical cancer, making it a cornerstone of modern cervical cancer prevention strategies [11–13]. However, the clinical and analytical accuracy of high-risk HPV (hrHPV) testing on self-collected samples as compared to CCS lacked validation studies. Hence, the VALHUDES (VALidation of HUman papillomavirus assays and collection DEvices for Self-samples and urine samples) protocol was designed to investigate the (relative) clinical and analytical performance of hrHPV testing on self-collected VSS and FVU using CSS as comparator [14]. As such, this data could aid in the recommendation of cervical cancer screening programs. Moreover, a standardised protocol for the validation of hrHPV tests in self-samples should facilitate the implementation of self-samples in the clinic. Meta-analyses have described the overall non-inferiority of target amplification-based tests in VSS as compared to CCS [4,7,15]. Yet, this should also be established for each assay, sampling and extraction method individually prior to their potential applications. Previous VALHUDES publications report on the similar clinical accuracy of the RealTi*me* High-Risk HPV assay (Abbot Molecular Diagnostics, Des Plaines, IL) [16,17], Alinity m HR HPV Assay (Abbot Molecular Diagnostics, Des Plaines, IL) [18], Allplex HPV HR Detection (Seegene, Seoul, South Korea [Latsuzbaia et al., manuscript submitted]), BD Onclarity HPV Assay (BD Diagnostics, Sparks, MD) [19,20], Xpert HPV assay (Xpert HPV; Cepheid, Sunnyvale, CA) [21], Liferiver HarmoniaHPV and VenusHPV assays (Shanghai ZJ Bio-Tech Co. Ltd., China) [22], and Roche Cobas 4800 and 6800 (Roche Diagnostics, Basel, Switzerland) [23] for self-collected samples compared to CCS.

This report aims to evaluate the relative clinical and analytical accuracy of the EUROArray HPV test (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany) on VSS and FVU compared to CCS. EUROArray was previously found partially validated on CCS according to the VALGENT protocol and after cut-off optimisation it fulfilled all necessary criteria [24,25]. This is the first time that the EUROArray HPV assay is tested on self-collected samples and compared to CCS as reference sample.

## 2. Materials and methods

### 2.1. Study design

The VALHUDES protocol (NCT03064087) has previously been described in detail [14,16]. In brief, patients who were referred for colposcopy because of aberrant cervical test results and/or HPV infections were invited to participate. In total, five colposcopy centres participated: the University Hospitals of Antwerp (UZA), Brussels (UZ Brussels), Ghent (UZ Ghent) and Liège (CHU de Liège) and the General Regional Hospital Heilig Hart Tienen (RZ Tienen). Patients were asked to collect four self-samples. This included two FVU samples collected with the 20 mL Colli-Pee™ (Colli-Pee, a product from DNA Genotek Inc., Ottawa, Canada) prefilled with 6.7 mL urine conservation medium (UCM, DNA Genotek Inc., Ottawa, Canada) one day before colposcopy visit. In addition, two VSS were collected at the colposcopy centre. First using the multi-Collect swab (Abbott Molecular Diagnostics, Des Plaines, IL), followed by either the Evalyn Brush (Rovers Medical Devices, B.V., Oss, The Netherlands) or Qvintip (Aprovix, Stockholm, Sweden). In this study, first FVU and second VSS (Evalyn Brush/Qvintip) were tested. The dry vaginal swabs were resuspended in 20 mL PreservCyt solution (Hologic Marlborough, MA, USA) upon arrival at the lab (Algemeen Medisch Laboratorium, AML, Antwerp, Belgium). Furthermore, a CCS was collected prior to colposcopy using a Cervex Brush (Rovers Medical Devices, B.V., Oss, NL) and was subsequently resuspended in 20 mL PreservCyt at the colposcopy centres. The FVU samples were aliquoted and stored at −80 °C at the University of Antwerp (Biobank Antwerp, Antwerp Belgium; ID: BE 71,030,031,000) [26]. VSS and CCS were stored at 4 °C in the lab (AML) for up to three months prior to aliquoting and storage at −80 °C (Biobank, BB190002).

The study was approved by the central Ethics Committee of the Antwerp University Hospital and University of Antwerp (B300201733869) as well as by the local Ethics Committees of the participating colposcopy centres. The study was conducted in accordance with the Declaration of Helsinki and written informed consent was obtained from all study participants prior to inclusion.

### 2.2. HPV testing

The EUROArray test at AML was used for HPV testing. It detects 30 HPV types, determining positivity for each of the types using type-specific confidential immunofluorescence unit cut-offs. The cut-offs have been established for CCS using validated devices [24]. Fourteen of the detected types are considered high-risk (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68), while HPV6, 11, 26, 40, 42, 43, 44, 53, 54, 61, 70, 72, 73, 81, 82, and 89 are considered low-risk HPV genotypes. For the accuracy estimation only high-risk HPV results were considered.

From all samples, an aliquot of 0.7 mL was used for DNA extraction with the Abbott *m*2000 automated system (using 0.4 mL input volume). The DNA was eluted in 50 μL of which 5 μL DNA extract was used in the test system of the EUROArray. The assay contains multiplex PCR primers targeting the E6 and E7 oncogenes after which the PCR products are labelled with fluorescent dyes. Subsequently, the mixture is transferred on microarray BIOCHIPs slides, scanned and analysed using the EUROArrayScan software. Positivity rates for the human *HSP90AB1* gene are used as internal control to ensure validity of the tested samples. Samples negative for *HSP90AB1* are considered invalid.

In this study, the comparator test consisted of HPV testing on cervical samples, whereas the index test was HPV testing on self-samples. Furthermore, colposcopy and histology results were used as the reference standard. Patients with normal colposcopy observations, and thus without the need for biopsy, were classified as <CIN2. In case of multiple biopsies, the most severe outcome was used in the analysis.

### 2.3. Data analysis

Clinical accuracy was measured with 95 % confidence intervals (95 % CI). The relative clinical sensitivity (CIN2+ and CIN3) and specificity (<CIN2) of the EUROArray HPV test on self-samples was expressed as proportion compared to the clinical performance on CCS applying the McNemar test ($P_{McN}$). Differences are considered statistically significant when the confidence intervals do not include unity ($P_{McN} < 0.05$). Relative accuracy with 95 % CIs below 1.0 means that the EUROArray HPV test is more accurate on CCS than on self-samples, while values with 95 % CIs above 1.0 refer to greater accuracy measures in self-samples than in CCS. This is described in more detail in the VALHUDES protocol [14]. In addition, test agreement for both overall hrHPV results as well as for type-specific hrHPV results was calculated between the different sample types using Cohen's Kappa (κ). The following categories were applied: 0–0.20, poor; 0.21–0.40, fair; 0.41–0.60, moderate; 0.61–0.80, good; 0.81–1.00, excellent agreement.

## 3. Results

### 3.1. Study population

The study included 523 patients who were referred to colposcopy and signed informed consent to participate between 31/12/2017 and 02/01/2020 (Fig. 1). Of these, 24 were excluded due to incomplete sample sets, inadequate comparator test or other major protocol deviations regarding sample collection and processing [16,17]. In total, 499 paired self- and clinician-collected samples were obtained and tested with EUROArray. HPV results were valid for 491 matched VSS and CCS, and 494 matched FVU and CCS. In total 405 matched VSS were collected by participants with <CIN2 and 86 were CIN2+. For FVU, 406 participants had <CIN2 and 88 CIN2+.

### 3.2. Clinical performance of the full HPV genotyping assay

Absolute accuracy is summarised in Table 1 and Fig. 2. HrHPV testing using the EUROArray test was similarly sensitive to detect CIN2+ and CIN3 on VSS as compared to CCS (ratio, 0.96 [95 % CI: 0.91–1.02] and 0.95 [95 % CI: 0.87–1.05], resp.), Fig. 3 and Table 1. The specificity of VSS and CCS was also similar (ratio, 0.98 [95 % CI: 0.91–1.06]). Similar results were observed for FVU with relative clinical sensitivity of 0.96 (95 % CI: 0.90–1.03) for CIN2+ and 0.95 (95 % CI: 0.87–1.05) for CIN3. Likewise, relative specificity was not significantly different from unity, with a ratio of 0.94 (95 % CI: 0.85–1.04). Stratification

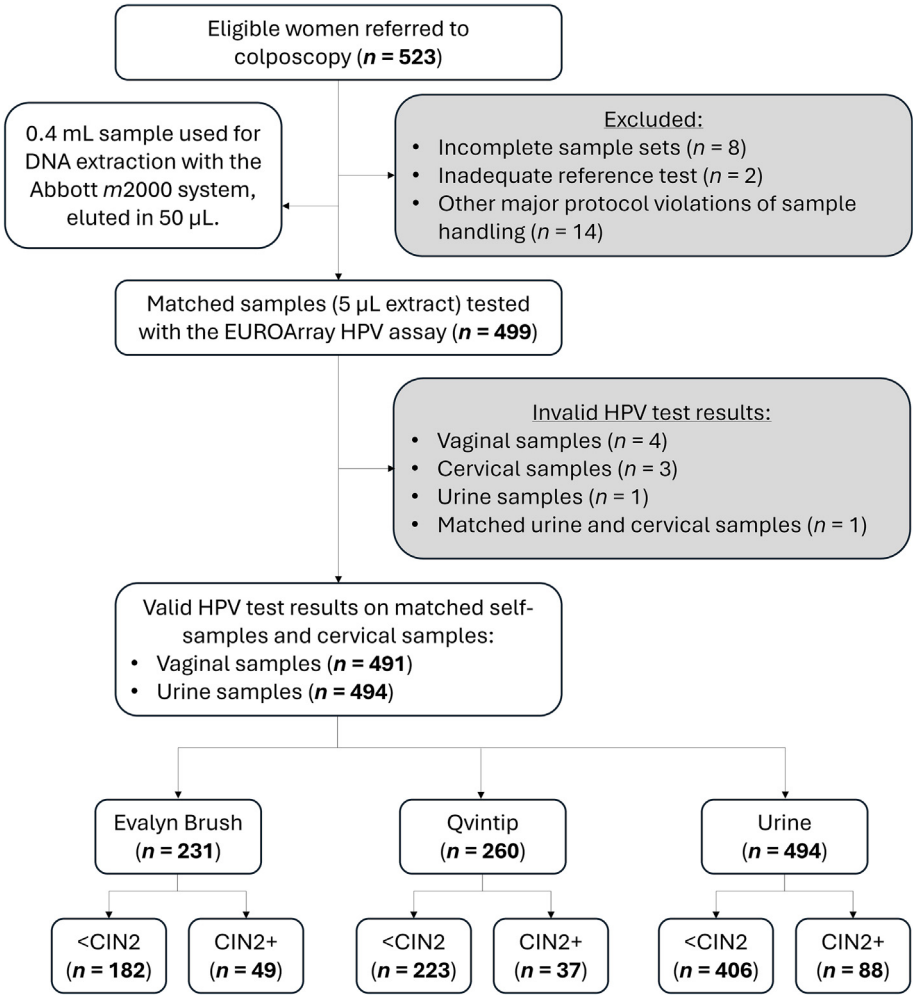


**Fig. 1.** Flowchart of the VALHUDES study analysed by EUROArray. Excluded samples are shown in grey. CIN; Cervical Intraepithelial Neoplasia.

**Table 1**
Clinical and Analytical Sensitivity and Specificity of the EUROArray HPV test on clinician-collected cervical samples (CCS), vaginal self-samples (VSS, Evalyn Brush and Qvintip combined) and first-void urine (FVU). *For the clinician-collected cervical samples (CCS), only the results for samples matched with FVU are shown, with similar results for CCS matched with VSS. CIN; cervical intraepithelial neoplasia.

| | Absolute clinical accuracy, in % [95 % CI], n/N | | | Relative clinical accuracy [95 % CI], $p_{McN}$ | | |
|---|---|---|---|---|---|---|
| Sample type | Sensitivity CIN2+ | Sensitivity CIN3 | Specificity < CIN2 | Sensitivity CIN2+ | Sensitivity CIN3 | Specificity < CIN2 |
| CCS* | 94.3 [87.2–98.1], 83/88 | 95.6 [84.9–99.5], 43/45 | 45.8 [40.9–50.8], 186/406 | – | – | – |
| VSS | 90.7 [82.5–95.9], 78/86 | 91.1 [78.8–97.5], 41/45 | 45.2 [40.3–50.2], 183/405 | 0.963 [0.911–1.018], 0.1797 | 0.953 [0.869–1.047], 0.3173 | 0.984 [0.909–1.065], 0.6858 |
| Evalyn Brush | 93.9 [83.1–98.7], 46/49 | 91.7 [73.0–99.0], 22/24 | 47.8 [40.4–55.3], 87/182 | 1.000 [0.942–1.062], 1.0000 | 1.000 [0.882–1.134], 1.0000 | 1.061 [0.954–1.180], 0.2752 |
| Qvintip | 86.5 [71.2–95.5], 32/37 | 90.5 [69.6–98.8], 19/21 | 43.0 [36.5–49.8], 127/223 | 0.914 [0.826–1.012], 0.0833 | 0.905 [0.788–1.104], 0.1573 | 0.923 [0.823–1.035], 0.1701 |
| FVU | 90.9 [82.9–96.0], 80/88 | 91.1 [78.8–97.5], 41/45 | 43.1 [38.2–48.1], 175/406 | 0.963 [0.904–1.027], 0.2568 | 0.953 [0.869–1.047], 0.3173 | 0.941 [0.852–1.039], 0.2273 |

by the vaginal sampling device showed slight differences (although not statistically significant) when separately compared with the CCS (relative clinical accuracy = 1.00 [95 % CI: 0.94–1.06] for Evalyn Brush and 0.91 [95 % CI: 0.83–1.01] for Qvintip). In addition, the relative clinical specificity for <CIN2 of EUROArray testing on the two vaginal brushes compared to the CCS was different although not significantly: higher than unity (1.06 [95 % CI: 0.95–1.18]) for Evalyn Brush and lower than unity (0.92 [95 % CI: 0.82–1.04]) for Qvintip. When directly comparing the two devices and expressing the accuracy of Evalyn Brush to that of Qvintip, the relative sensitivity is 1.09 (95 % CI: 0.94–1.26, $p = 0.24$) and relative specificity is 1.11 (95 % CI: 0.90–1.38, $p = 0.33$). While these differences are not significant, these results suggest the tendency of Evalyn Brush to outperform Qvintip.

### 3.3. Analytical performance

The hrHPV test concordance is shown in Table 2, and other (not high-risk) HPV test outcomes in **Table S1**. The concordance is stratified per sample type and device, and by disease status. Moderate to excellent test agreement was detected for overall hrHPV and hrHPV type-specific results, with κ-values ranging from 0.41 to 1.00. Generally, agreement was higher for VSS than FVU compared to CCS and for Evalyn Brush than Qvintip compared to CCS.

## 4. Discussion

The goal of the VALHUDES consortium is to generate clinical accuracy data regarding the accuracy of HPV testing on self-collected

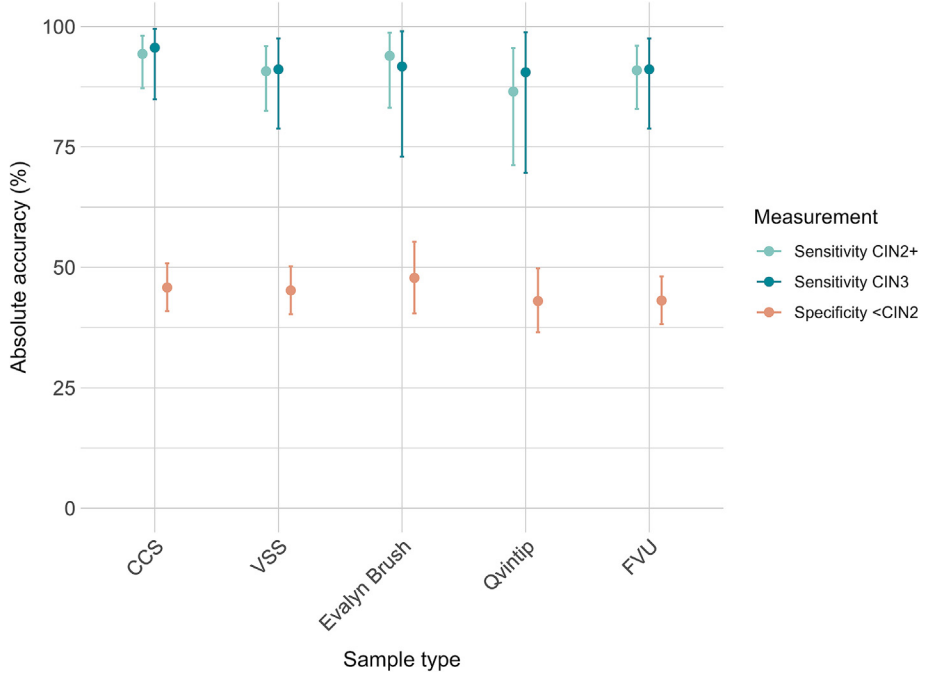


**Fig. 2.** Absolute clinical accuracy of EUROArray HPV testing on self- and clinician-collected samples. The absolute clinical accuracy of each sample type is shown. Vaginal self-samples (VSS) are stratified based on brush type. Absolute sensitivity for CIN2+ and CIN3 is shown with corresponding 95 % confidence interval (CI) as well as absolute specificity for <CIN2 with 95 % CI. For the clinician-collected cervical samples (CCS), only the results for samples matched with FVU are shown, with similar results for CCS matched with VSS. CIN; cervical intraepithelial neoplasia, CCS; clinician-collected cervical sample, VSS; vaginal self-sample (Evalyn Brush and Qvintip combined), FVU; first-void urine.

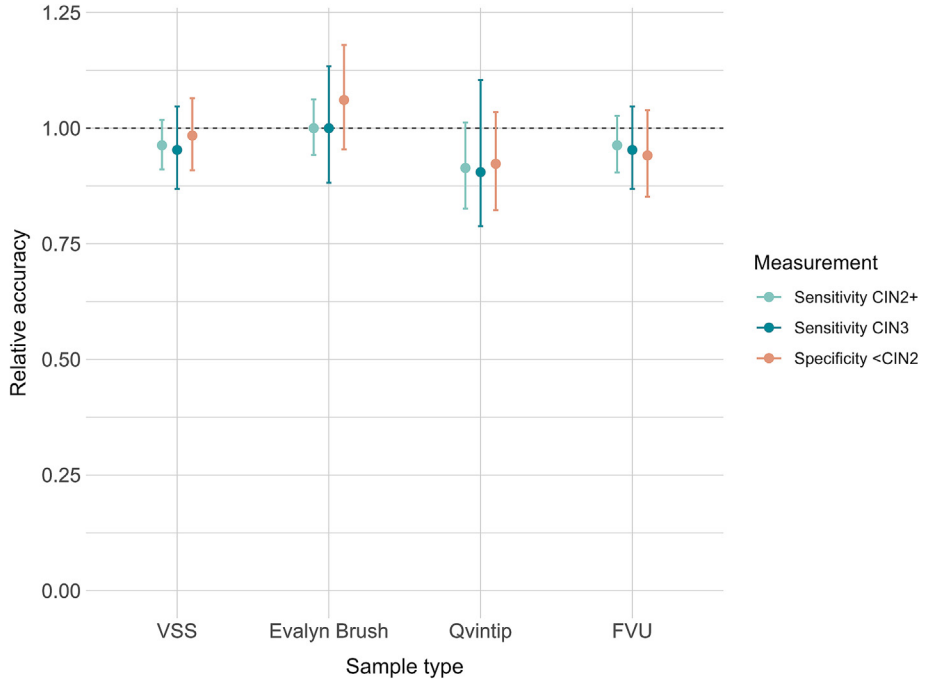


**Fig. 3.** Relative clinical accuracy of EUROArray HPV testing on self- vs. clinician-collected cervical samples. The relative clinical accuracy of each sample type is compared to clinician-collected cervical samples. Vaginal self-samples (VSS) are stratified based on brush type. Relative sensitivity for CIN2+ and CIN3 is shown with corresponding 95 % confidence interval (CI) as well as relative specificity for <CIN2 with 95 % CI. The dashed line indicates unity. CIN; cervical intraepithelial neoplasia, VSS; vaginal self-sample (Evalyn Brush and Qvintip combined), FVU; first-void urine.

samples using a wide range of validated HPV tests and extraction methods. With several HPV assays already published, this paper focuses on the functionality of the EUROArray HPV assay to distinguish people with and without cervical disease based on HPV test outcomes in FVU, VSS and CCS. The clinical accuracy of the EUROArray HPV assay, after Abbott *m*2000 DNA extraction, was found to be similar between self- and clinician-collected samples for both FVU and VSS.

The non-invasive aspect of the self-samples as well as the ability to collect them at home has demonstrated benefits for cervical screening purposes, particularly among populations that remain un(*der*)screened. Meta-analysis has shown that vaginal self-sampling [1] increases screening uptake and studies have also reported increased response rates with urine sampling [2,3]. For both sample types, directly sending self-sampling kits to participants' homes (mail-to-all approach) gained greater response than providing an opportunity to order kits (opt-in approach) [1–3]. Accordingly, self-sampling has been implemented in 17 countries worldwide as of 2021, either for the entire population or specifically for un(*der*)screened individuals [27].

In this study, both self-collected sample types showed no significant difference from cervical samples in terms of clinical accuracy. It should be noted that the study was not designed to detect differences between sampling devices and thus lacks sufficient power for this. Nevertheless, samples taken with the Evalyn Brush provided higher sensitivity and specificity than Qvintip samples, albeit not significant. A similar trend was observed in previous VALHUDES reports [17–19,21,22]. The brush heads of the Evalyn Brush and Qvintip devices consist of different materials. Where Evalyn Brushes are made of soft flexible brush hairs, Qvintip has a rigid brush head with notches. The Evalyn Brush also provides standardised collection by its wings and rotation system. When compared to a dry flocked swab and a wet dacron swab (WD), Qvintip was also found to be less sensitive for CIN2+ and CIN3+ detection [28]. As such, the collection of cervical secretions may be impacted by the device and hence also affect the HPV test results.

Another important consideration is the pre-analytical workflow. Differences in sample processing have been underreported but are impacting test outcomes [29–31]. Factors such as collection volume, suspension or conservation buffers, input volumes, extraction methods and sample storage conditions should be considered. In this study, the vaginal brushes were resuspended in 20 mL PreservCyt, reaching similar accuracy as cervical samples. Smaller volumes could concentrate the sample, increasing sensitivity but decreasing specificity. A reduction in specificity associated with lower resuspension volumes was previously observed in the Belgian and European VALHUDES studies [18,32]. As such, the clinical accuracy not only evaluates the HPV test, but the entire pre-analytical workflow as well. Moreover, the clinical accuracy of FVU with UCM was also not significantly different from cervical samples. Rohner et al. found lower sensitivity compared to cervical samples when using FVU collected without Colli-Pee devices, illustrating the importance of standardised collection devices [33]. Furthermore, the addition of conservation buffers is recommended to avoid degradation of viral DNA by nucleases [9,30]. The combination of the pre-analytical process described above with the EUROArray HPV test demonstrated similar clinical accuracy on self-collected samples and CCS.

The EUROArray HPV test is considered partially validated according to the VALGENT protocol due to its inferior sensitivity compared to Hybrid Capture 2 using previously established cut-offs. Yet, cut-off optimisation for HPV16 increased the sensitivity and led to the acceptance of the test for cervical cancer screening by fulfilling the international requirements [24,25]. Accordingly, this optimised cut-off was applied in this report. Previous VALHUDES reports also used cut-off optimisation to reach similar performance for self- and clinician-collected samples, but for the EUROArray HPV test this was not necessary, and current cut-offs established for cervical samples were found sufficiently accurate. The EUROArray provides individual genotyping results for 14 hrHPV types and 16 other HPV types. According to the International Agency for Research on Cancer (IARC), HPV66 and 68 are not seen as high-risk anymore, but rather possibly and probably carcinogenic, respectively [34,35]. Yet, they are referred to as high-risk in the assay. Currently, positivity of one of the 14 hrHPV types is considered for risk

**Table 2**
hrHPV test concordance (κ [95 % confidence interval (CI)]) between clinician-collected cervical samples and self-samples, overall and by disease status. κ 0.00–0.20 Poor; 0.21–0.40 Fair; 0.41–0.60 Moderate; 0.61–0.80 Good; 0.81–1.00 Excellent agreement. CCS; clinician-collected cervical samples, FVU; first-void urine, VSS; vaginal self-samples, CIN; cervical intraepithelial neoplasia.

| | FVU test result | | | | | | | | | | | | VSS test result | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ALL ($n = 494$) | | | | CIN2+ ($n = 88$) | | | | <CIN2 ($n = 406$) | | | | ALL ($n = 491$) | | | | CIN2+ ($n = 86$) | | | | <CIN2 ($n = 405$) | | | |
| HPV type / Comparator result (CCS) | + | − | Concordance (%) | κ [95 % CI] | + | − | Concordance (%) | κ [95 % CI] | + | − | Concordance (%) | κ [95 % CI] | + | − | Concordance (%) | κ [95 % CI] | + | − | Concordance (%) | κ [95 % CI] | + | − | Concordance (%) | κ [95 % CI] |
| hrHPV | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 262 | 41 | | 0.613 | 78 | 5 | | 0.421 | 184 | 36 | | 0.586 | 270 | 30 | | 0.743 | 77 | 4 | | 0.586 | 193 | 26 | | 0.726 |
| − | 49 | 142 | 81.8 | [0.541–0.685] | 2 | 3 | 92.1 | [0.071–0.771] | 47 | 139 | 79.6 | [0.507–0.666] | 30 | 161 | 87.8 | [0.682–0.804] | 1 | 4 | 94.2 | [0.260–0.911] | 29 | 157 | 86.4 | [0.659–0.793] |
| HPV16 | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 69 | 13 | | 0.553 | 38 | 2 | | 0.730 | 31 | 11 | | 0.411 | 69 | 14 | | 0.743 | 38 | 1 | | 0.883 | 31 | 12 | | 0.615 |
| − | 63 | 349 | 84.6 | [0.467–0.640] | 10 | 38 | 86.4 | [0.590–0.869] | 53 | 311 | 84.2 | [0.297–0.525] | 23 | 385 | 92.5 | [0.664–0.821] | 3 | 43 | 94.2 | [0.784–0.983] | 20 | 342 | 92.1 | [0.494–0.737] |
| HPV18 | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 13 | 8 | | 0.544 | 3 | 0 | | 0.651 | 10 | 8 | | 0.519 | 15 | 4 | | 0.803 | 3 | 0 | | 1.000 | 12 | 4 | | 0.765 |
| − | 12 | 461 | 96 | [0.367–0.721] | 3 | 82 | 96.6 | [0.287–1.000] | 9 | 379 | 98.3 | [0.318–0.719] | 3 | 469 | 98.6 | [0.661–0.946] | 0 | 83 | 100 | [1.000–1.000] | 3 | 386 | 98.3 | [0.597–0.933] |
| HPV31 | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 46 | 17 | | 0.698 | 15 | 7 | | 0.677 | 31 | 10 | | 0.698 | 46 | 16 | | 0.766 | 15 | 6 | | 0.791 | 31 | 10 | | 0.750 |
| − | 16 | 415 | 93.3 | [0.601–0.795] | 3 | 63 | 88.6 | [0.494–0.861] | 13 | 352 | 94.3 | [0.582–0.814] | 8 | 421 | 95.1 | [0.676–0.855] | 0 | 65 | 93 | [0.633–0.949] | 8 | 356 | 95.6 | [0.640–0.861] |
| HPV33 | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 14 | 8 | | 0.604 | 3 | 2 | | 0.465 | 11 | 6 | | 0.653 | 20 | 2 | | 0.825 | 4 | 1 | | 0.709 | 16 | 1 | | 0.858 |
| − | 9 | 463 | 96.6 | [0.432–0.776] | 4 | 79 | 93.2 | [0.103–0.826] | 5 | 384 | 97.3 | [0.461–0.844] | 6 | 463 | 98.4 | [0.706–0.944] | 2 | 79 | 96.5 | [0.396–1.000] | 4 | 384 | 98.8 | [0.736–0.981] |
| HPV35 | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 10 | 2 | | 0.734 | 4 | 1 | | 0.788 | 6 | 1 | | 0.700 | 8 | 4 | | 0.688 | 4 | 1 | | 0.883 | 4 | 3 | | 0.564 |
| − | 5 | 477 | 98.6 | [0.544–0.923] | 1 | 82 | 97.7 | [0.503–1.000] | 4 | 395 | 98.8 | [0.450–0.950] | 3 | 476 | 98.6 | [0.470–0.907] | 0 | 81 | 98.8 | [0.656–1.000] | 3 | 395 | 98.5 | [0.250–0.878] |
| HPV39 | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 23 | 11 | | 0.631 | 8 | 1 | | 0.690 | 15 | 10 | | 0.601 | 24 | 8 | | 0.759 | 8 | 0 | | 0.935 | 16 | 8 | | 0.694 |
| − | 13 | 447 | 95.1 | [0.495–0.767] | 5 | 74 | 93.2 | [0.459–0.920] | 8 | 373 | 95.6 | [0.434–0.769] | 6 | 453 | 97.2 | [0.637–0.881] | 1 | 77 | 98.8 | [0.808–1.000] | 5 | 376 | 96.8 | [0.537–0.851] |
| HPV45 | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 12 | 7 | | 0.600 | 3 | 2 | | 0.649 | 9 | 5 | | 0.585 | 14 | 5 | | 0.817 | 4 | 1 | | 0.788 | 10 | 4 | | 0.828 |
| − | 8 | 467 | 97 | [0.415–0.785] | 1 | 82 | 96.6 | [0.280–1.000] | 7 | 385 | 97 | [0.372–0.798] | 1 | 471 | 98.8 | [0.674–0.960] | 1 | 80 | 97.7 | [0.502–1.000] | 0 | 391 | 99 | [0.664–0.993] |
| HPV51 | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 23 | 14 | | 0.591 | 6 | 3 | | 0.549 | 17 | 11 | | 0.603 | 25 | 11 | | 0.670 | 7 | 2 | | 0.704 | 18 | 9 | | 0.657 |
| − | 14 | 443 | 94.3 | [0.454–0.728] | 5 | 74 | 90.9 | [0.273–0.826] | 9 | 369 | 95.1 | [0.445–0.762] | 11 | 444 | 95.5 | [0.541–0.799] | 3 | 74 | 94.2 | [0.460–0.948] | 8 | 370 | 95.8 | [0.505–0.809] |
| HPV52 | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 33 | 19 | | 0.692 | 3 | 5 | | 0.421 | 30 | 14 | | 0.745 | 44 | 8 | | 0.876 | 7 | 1 | | 0.862 | 37 | 7 | | 0.879 |
| − | 6 | 436 | 94.9 | [0.579–0.805] | 2 | 78 | 92.1 | [0.071–0.771] | 4 | 358 | 95.6 | [0.633–0.857] | 3 | 436 | 97.8 | [0.805–0.948] | 1 | 77 | 97.7 | [0.675–1.000] | 2 | 359 | 97.8 | [0.802–0.957] |
| HPV56 | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 26 | 10 | | 0.735 | 7 | 1 | | 0.663 | 19 | 9 | | 0.761 | 31 | 6 | | 0.825 | 7 | 1 | | 0.804 | 24 | 5 | | 0.830 |
| − | 7 | 451 | 96.6 | [0.615–0.855] | 5 | 75 | 93.2 | [0.415–0.911] | 2 | 376 | 97.3 | [0.626–0.897] | 6 | 448 | 97.6 | [0.728–0.922] | 2 | 76 | 96.5 | [0.590–1.000] | 4 | 372 | 97.8 | [0.722–0.939] |
| HPV58 | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 24 | 8 | | 0.696 | 5 | 1 | | 0.69 | 19 | 7 | | 0.697 | 28 | 4 | | 0.881 | 6 | 0 | | 0.917 | 22 | 4 | | 0.872 |
| − | 11 | 451 | 96.2 | [0.567–0.825] | 3 | 79 | 95.5 | [0.405–0.975] | 8 | 372 | 96.3 | [0.552–0.842] | 3 | 456 | 98.6 | [0.794–0.968] | 1 | 79 | 98.8 | [0.755–1.000] | 2 | 377 | 98.5 | [0.771–0.973] |
| HPV59 | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 17 | 9 | | 0.621 | 3 | 2 | | 0.576 | 14 | 7 | | 0.632 | 21 | 5 | | 0.781 | 4 | 1 | | 0.883 | 17 | 4 | | 0.760 |
| − | 10 | 458 | 96.2 | [0.464–0.778] | 2 | 81 | 95.5 | [0.201–0.951] | 8 | 377 | 96.3 | [0.459–0.804] | 6 | 459 | 97.8 | [0.655–0.906] | 0 | 81 | 98.8 | [0.656–1.000] | 6 | 378 | 97.5 | [0.616–0.903] |
| HPV66 | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 18 | 4 | | 0.81 | 4 | 0 | | 1 | 14 | 4 | | 0.767 | 20 | 1 | | 0.884 | 3 | 1 | | 0.851 | 17 | 0 | | 0.890 |
| − | 4 | 468 | 98.4 | [0.681–0.938] | 0 | 84 | 100 | [1.000–1.000] | 4 | 384 | 98 | [0.612–0.923] | 4 | 466 | 99 | [0.783–0.984] | 0 | 82 | 98.8 | [0.565–1.000] | 4 | 384 | 99 | [0.783–0.997] |
| HPV68 | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 16 | 7 | | 0.681 | 5 | 1 | | 0.821 | 11 | 6 | | 0.632 | 16 | 7 | | 0.697 | 5 | 1 | | 0.75 | 11 | 6 | | 0.675 |
| − | 7 | 464 | 97.2 | [0.523–0.838] | 1 | 81 | 97.7 | [0.579–1.000] | 6 | 383 | 97 | [0.439–0.825] | 6 | 462 | 97.4 | [0.541–0.853] | 2 | 78 | 96.5 | [0.480–1.000] | 4 | 384 | 97.5 | [0.485–0.864] |

| | Evalyn Brush test result | | | | | | | | | | | | Qvintip test result | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ALL ($n$ = 231) | | | | CIN2+ (n = 49) | | | | <CIN2 ($n$ = 182) | | | | ALL ($n$ = 260) | | | | CIN2+ ($n$ = 37) | | | | <CIN2 ($n$ = 223) | | | |
| HPV type / Comparator result (CCS) | + | − | Concordance (%) | κ [95 % CI] | + | − | Concordance (%) | κ [95 % CI] | + | − | Concordance (%) | κ [95 % CI] | + | − | Concordance (%) | κ [95 % CI] | + | − | Concordance (%) | κ [95 % CI] | + | − | Concordance (%) | κ [95 % CI] |
| hrHPV | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 132 | 14 | | 0.789 | 45 | 1 | | 0.645 | 87 | 13 | | 0.768 | 138 | 16 | | 0.703 | 32 | 3 | | 0.536 | 106 | 13 | | 0.692 |
| − | 9 | 76 | 90 | [0.707–0.870] | 1 | 2 | 95.9 | [0.188–1.000] | 8 | 74 | 88.5 | [0.675–0.861] | 21 | 85 | 85.8 | [0.615–0.791] | 0 | 2 | 91.9 | [0.089–0.982] | 21 | 83 | 84.8 | [0.597–0.787] |
| HPV16 | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 41 | 5 | | 0.840 | 27 | 1 | | 0.874 | 14 | 4 | | 0.729 | 28 | 9 | | 0.635 | 11 | 1 | | 0.877 | 17 | 8 | | 0.538 |
| − | 7 | 178 | 94.8 | [0.752–0.928] | 2 | 19 | 93.9 | [0.737–1.000] | 5 | 159 | 95.1 | [0.561–0.897] | 16 | 207 | 90.4 | [0.504–0.766] | 1 | 24 | 94.6 | [0.711–1.000] | 15 | 183 | 89.7 | [0.373–0.704] |
| HPV18 | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 11 | 3 | | 0.837 | 1 | 0 | | 1.000 | 10 | 3 | | 0.822 | 4 | 1 | | 0.721 | 2 | 0 | | 1.000 | 2 | 1 | | 0.565 |
| − | 1 | 216 | 98.3 | [0.681–0.994] | 0 | 48 | 100 | [1.000–1.000] | 1 | 168 | 97.8 | [0.651–0.992] | 2 | 253 | 98.9 | [0.420–1.000] | 0 | 35 | 100 | [1.000–1.000] | 2 | 218 | 98.7 | [0.122–1.000] |
| HPV31 | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 16 | 9 | | 0.719 | 6 | 3 | | 0.766 | 10 | 6 | | 0.691 | 30 | 7 | | 0.793 | 9 | 3 | | 0.802 | 21 | 4 | | 0.782 |
| − | 2 | 204 | 95.2 | [0.562–0.876] | 0 | 40 | 93.9 | [0.516–1.000] | 2 | 164 | 95.6 | [0.490–0.892] | 6 | 217 | 95 | [0.684–0.901] | 0 | 25 | 91.9 | [0.592–1.000] | 6 | 192 | 95.5 | [0.653–0.912] |
| HPV33 | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 7 | 1 | | 0.769 | 1 | 1 | | 0.657 | 6 | 0 | | 0.792 | 13 | 1 | | 0.859 | 3 | 0 | | 0.722 | 10 | 1 | | 0.904 |
| − | 3 | 220 | 98.3 | [0.550–0.988] | 0 | 47 | 98 | [0.033–1.000] | 3 | 173 | 98.4 | [0.563–1.000] | 3 | 243 | 98.5 | [0.722–0.995] | 2 | 32 | 94.6 | [0.362–1.000] | 1 | 211 | 99.1 | [0.773–1.000] |
| HPV35 | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 4 | 1 | | 0.887 | 2 | 0 | | 1.000 | 2 | 1 | | 0.797 | 4 | 3 | | 0.560 | 2 | 1 | | 0.786 | 2 | 2 | | 0.433 |
| − | 0 | 226 | 99.6 | [0.667–1.000] | 0 | 47 | 100 | [1.000–1.000] | 0 | 179 | 99.5 | [0.409–1.000] | 3 | 250 | 97.7 | [0.244–0.875] | 0 | 34 | 97.3 | [0.382–1.000] | 3 | 216 | 97.8 | [0.023–0.843] |
| HPV39 | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 9 | 2 | | 0.809 | 3 | 0 | | 1.000 | 6 | 2 | | 0.739 | 15 | 6 | | 0.729 | 5 | 0 | | 0.893 | 10 | 6 | | 0.668 |
| − | 2 | 218 | 98.3 | [0.626–0.992] | 0 | 46 | 100 | [1.000–1.000] | 2 | 172 | 97.8 | [0.493–0.984] | 4 | 235 | 96.2 | [0.569–0.889] | 1 | 31 | 97.3 | [0.688–1.000] | 3 | 204 | 96 | [0.466–0.871] |
| HPV45 | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 3 | 2 | | 0.66 | 2 | 0 | | 0.79 | 1 | 2 | | 0.496 | 11 | 3 | | 0.874 | 2 | 1 | | 0.786 | 9 | 2 | | 0.895 |
| − | 1 | 225 | 98.7 | [0.299–1.000] | 1 | 46 | 98 | [0.391–1.000] | 0 | 179 | 98.9 | [−0.104–1.000] | 0 | 246 | 98.9 | [0.733–1.000] | 0 | 34 | 97.3 | [0.382–1.000] | 0 | 212 | 99.1 | [0.752–1.000] |
| HPV51 | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 14 | 5 | | 0.713 | 4 | 0 | | 0.778 | 10 | 5 | | 0.691 | 11 | 6 | | 0.622 | 3 | 2 | | 0.621 | 8 | 4 | | 0.619 |
| − | 5 | 207 | 95.7 | [0.545–0.882] | 2 | 43 | 95.9 | [0.485–1.000] | 3 | 164 | 95.6 | [0.489–0.892] | 6 | 237 | 95.4 | [0.426–0.819] | 1 | 31 | 91.9 | [0.230–1.000] | 5 | 206 | 96 | [0.390–0.848] |
| HPV52 | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 23 | 3 | | 0.932 | 3 | 1 | | 0.846 | 20 | 2 | | 0.946 | 21 | 5 | | 0.823 | 4 | 0 | | 0.874 | 17 | 5 | | 0.812 |
| − | 0 | 205 | 98.7 | [0.855–1.000] | 0 | 45 | 98 | [0.552–1.000] | 0 | 16 | 98.9 | [0.872–1.000] | 3 | 231 | 96.9 | [0.704–0.942] | 1 | 32 | 97.3 | [0.632–1.000] | 2 | 199 | 96.9 | [0.677–0.947] |
| HPV56 | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 15 | 3 | | 0.873 | 5 | 0 | | 1.000 | 10 | 3 | | 0.822 | 16 | 3 | | 0.783 | 2 | 1 | | 0.528 | 14 | 2 | | 0.836 |
| − | 1 | 212 | 98.3 | [0.751–0.996] | 0 | 44 | 100 | [1.000–1.000] | 1 | 168 | 97.8 | [0.651–0.992] | 5 | 236 | 96.9 | [0.638–0.929] | 2 | 32 | 91.9 | [0.060–0.995] | 3 | 204 | 97.8 | [0.696–0.977] |
| HPV58 | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 15 | 2 | | 0.873 | 5 | 0 | | 1.000 | 10 | 2 | | 0.822 | 13 | 2 | | 0.890 | 1 | 0 | | 0.654 | 12 | 2 | | 0.918 |
| − | 2 | 212 | 98.3 | [0.750–0.996] | 0 | 44 | 100 | [1.000–1.000] | 2 | 168 | 97.8 | [0.651–0.992] | 1 | 244 | 98.9 | [0.768–1.000] | 1 | 35 | 97.3 | [0.027–1.000] | 0 | 209 | 99.1 | [0.806–1.000] |
| HPV59 | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 10 | 2 | | 0.824 | 3 | 0 | | 1.000 | 7 | 2 | | 0.766 | 11 | 3 | | 0.744 | 1 | 1 | | 0.654 | 10 | 2 | | 0.755 |
| − | 2 | 217 | 98.3 | [0.656–0.993] | 0 | 46 | 100 | [1.000–1.000] | 2 | 171 | 97.8 | [0.545–0.988] | 4 | 242 | 97.3 | [0.563–0.926] | 0 | 35 | 97.3 | [0.027–1.000] | 4 | 207 | 97.3 | [0.567–0.944] |
| HPV66 | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 7 | 0 | | 0.769 | 1 | 0 | | 1.000 | 6 | 0 | | 0.739 | 13 | 1 | | 0.961 | 2 | 1 | | 0.786 | 11 | 0 | | 1.000 |
| − | 4 | 220 | 98.3 | [0.551–0.987] | 0 | 48 | 100 | [1.000–1.000] | 4 | 172 | 97.8 | [0.495–0.983] | 0 | 246 | 99.6 | [0.885–1.000] | 0 | 34 | 97.3 | [0.382–1.000] | 0 | 212 | 100 | [1.000–1.000] |
| HPV68 | | | | | | | | | | | | | | | | | | | | | | | | |
| + | 5 | 5 | | 0.573 | 2 | 1 | | 0.645 | 3 | 4 | | 0.532 | 11 | 2 | | 0.774 | 3 | 0 | | 0.843 | 8 | 2 | | 0.750 |
| − | 2 | 219 | 97 | [0.288–0.858] | 1 | 45 | 95.9 | [0.188–1.000] | 1 | 174 | 97.3 | [0.172–0.893] | 4 | 243 | 97.7 | [0.598–0.949] | 1 | 33 | 97.3 | [0.542–1.000] | 3 | 210 | 97.8 | [0.539–0.961] |

stratification. Hence, results of the other types have less clinical significance, but can be used for epidemiological studies as well as vaccine impact monitoring and prevalence studies. Concordance between the hrHPV type results varied depending on sample type, device and disease group. The overall hrHPV concordance between self- and clinician samples was higher for VSS than FVU ($\kappa = 0.74$ vs. 0.61), and higher for Evalyn Brush than Qvintip ($\kappa = 0.79$ vs. 0.70). κ-values were higher for <CIN2 versus CIN2+ for some less carcinogenic genotypes. Yet, for HPV16 and 18 the opposite is observed, with higher concordance for CIN2+ than <CIN2, regardless of sample type. This could be caused by the significant role these types have in the development of cervical disease, being responsible for 77 % of cervical cancers [36]. The difference in pathogenicity between the HPV types has led to its implementation in screening and triage strategies, with HPV16 positive people with a cervix often directly referred to colposcopy without prior cytological evaluation. Considering the lower positive predictive values of HPV39, 51, 56 and 59, it has been suggested these may not be a priority in screening programs, leading to unnecessary referrals [36]. Within the VALHUDES study, EUROArray is one of the four tests providing individual genotyping results, together with Liferiver VenusHPV, Seegene Allplex (manuscript in prep) and Riatol qPCR (manuscript in prep). The other tests provide only partial genotyping results. Considering the value of HPV genotyping in risk stratification, extended or full genotyping is beneficial for triage of HPV-positive individuals with a cervix.

Previous meta-analyses have reported greater accuracy of target-amplification over signal amplification based methods for the detection of HPV in self-samples [4,7]. Following this principle, EUROArray results are acquired through PCR amplification of the target, after which the fluorescent signal is measured. Similar to the findings of these meta-analyses, EUROArray HPV testing demonstrated equal accuracy on both self- and clinician-collected samples. Moreover, EUROArray hrHPV detection on CCS was in good or very good agreement with other commercially available hrHPV assays (Aptima, $\kappa = 0.79$; Anyplex II HPV28, $\kappa = 0.84$; Hybrid Capture, $\kappa = 0.62$; Cobas, $\kappa = 0.81$; Amplicor, $\kappa = 0.68$; and Linear Array $\kappa = 0.77$) [37–39]. Although the EUROArray has slightly lower analytical sensitivity than Linear Array, the clinical accuracy was similar to all previously mentioned assays except Aptima for which clinical performance was not determined [37–39]. Clinical sensitivity and specificity of EUROArray on CCS in follow-up settings was in accordance with the other tests, measuring 86 % sensitivity for CIN2+ at 71 % specificity for <CIN2 [38]. The current study demonstrates higher clinical sensitivity (94 %) and lower specificity (46 %). The low specificity in both studies can be explained by the fact that participants were recruited due to previous cervical abnormalities or HPV infections leading to high HPV positivity rates.

Previous VALHUDES publications describe the limitations of the study in depth. The major limitation is the referral setting itself, which has the advantage of faster recruiting sufficient participants with cervical disease but may influence accuracy measures. While relative accuracy is considered a robust metric as all samples are affected similarly, it has recently been suggested that relative sensitivity is expected to be optimistic [40]. Yet, these results provide a promising first step in the clinical acceptance of the EUROArray on self-samples and further research in screening settings is recommended.

In conclusion, this report shows that there are no significant differences in the clinical performance of the EUROArray HPV assay on FVU or VSS versus CCS. Although the clinical accuracy of Evalyn Brush samples is higher than that of Qvintip samples, this difference is not statistically significant. These results strengthen the existing evidence regarding the accuracy of different HPV tests in self-collected samples, supporting the transition to clinical implementation.

## CRediT authorship contribution statement

**Eef van den Borst:** Writing – review & editing, Writing – original draft, Visualization. **Davy Vanden Broeck:** Writing – review & editing, Validation, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Philippe De Sutter:** Writing – review & editing, Investigation. **Gilbert Donders:** Writing – review & editing, Investigation. **Jean Doyen:** Writing – review & editing, Investigation. **Wiebren Tjalma:** Writing – review & editing, Investigation, Conceptualization. **Steven Weyers:** Writing – review & editing, Investigation. **Marc Arbyn:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Severien Van Keer:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Ardashel Latsuzbaia:** Writing – review & editing, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis.

## Ethics statement

The VALHUDES trial (NCT03064087) was approved by the central Ethics Committee of the University Hospital of Antwerp/University of Antwerp (B300201733869) and the local Ethics Committees of all the other involved centers. The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all study participants before enrolment.

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## Declaration of competing interest

The VALHUDES project is a researcher-induced study, designed by Sciensano (Principal Investigator; Brussels, Belgium), CEV (University of Antwerp, Antwerp, Belgium), and AML (Antwerp, Belgium). Manufacturers of HPV assays and devices can participate in the VALHUDES framework contributing equipment for laboratory testing and financial support for statistical analysis under the condition of accepting independent publication of results. Manufacturers collaborating in VALHUDES to date are Abbott, Becton Dickinson, Cepheid, Euroimmun, Liferiver, Novosanis, Roche, and Seegene. The study group received sample collection devices from Rovers Medical Devices B.V. (Oss, The Netherlands) and Aprovix AB (Solna, Sweden). The funders had no role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication. All funds are handled and managed by the University of Antwerp. The University of Antwerp received payment for participation of Severien Van Keer in an Advisory Board of Novosanis (wholly owned subsidiary of OraSure Technologies, Inc., Pennsylvania, USA). The University of Antwerp obtained unrestricted educational grants from Becton Dickinson, Hologic, and Roche. Davy Vanden Broeck is employed by AML (Antwerp, Belgium), part of the National Reference Centre HPV, a private lab performing routine cervical cytology and HPV testing.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2025.08.018.

## Data availability

Final study datasets generated by VALHUDES will be stored locally and securely at Sciensano. Anonymized data will be available by request to the corresponding author on a case-by-case basis pending approval from the information security coordinator at Sciensano and involved study partners.

## References

[1] S. Costa, B. Verberckmoes, P.E. Castle, M. Arbyn, Offering HPV self-sampling kits: an updated meta-analysis of the effectiveness of strategies to increase participation in cervical cancer screening, Br. J. Cancer 128 (5) (Mar 2023) 805–813, doi:10.1038/s41416-022-02094-w.
[2] C. Lefeuvre, A. Pivert, H.L. Guillou-Guillemette, et al., Urinary HPV DNA testing as a tool for cervical cancer screening in women who are reluctant to have a pap smear in France, J. Inf. Secur. 81 (2) (Aug 2020) 248–254, doi:10.1016/j.jinf.2020.05.006.
[3] A. Ducancelle, J. Reiser, A. Pivert, H. Le Guillou-Guillemette, A.S. Le Duc-Banaszuk, F. Lunel-Fabiani, Home-based urinary HPV DNA testing in women who do not attend cervical cancer screening clinics, J. Inf. Secur. 71 (3) (Sep 2015) 377–384, doi:10.1016/j.jinf.2015.05.001.
[4] M. Arbyn, S.B. Smith, S. Temin, et al., Detecting cervical precancer and reaching underscreened women by using HPV testing on self samples: updated meta-analyses, BMJ 363 (2018), k4823, doi:10.1136/bmj.k4823.
[5] World Health Organization, Global Strategy to Accelerate the Elimination of Cervical Cancer as a Public Health Problem, World Health Organization, 2020 56.
[6] N. Pathak, J. Dodds, J. Zamora, K. Khan, Accuracy of urinary human papillomavirus testing for presence of cervical HPV: systematic review and meta-analysis, BMJ 349 (Sep 16 2014), g5264, doi:10.1136/bmj.g5264.
[7] M. Arbyn, P.E. Castle, M. Schiffman, N. Wentzensen, B. Heckman-Stoddard, V.V. Sahasrabuddhe, Meta-analysis of agreement/concordance statistics in studies comparing self- vs clinician-collected samples for HPV testing in cervical cancer screening, Int. J. Cancer 151 (2) (Jul 15 2022) 308–312, doi:10.1002/ijc.33967.
[8] A. Vorsters, I. Micalessi, J. Bilcke, M. Ieven, J. Bogers, P. Van Damme, Detection of human papillomavirus DNA in urine. A review of the literature, Eur. J. Clin. Microbiol. Infect. Dis. 31 (5) (May 2012) 627–640, doi:10.1007/s10096-011-1358-z.
[9] A. Vorsters, J. Van den Bergh, I. Micalessi, et al., Optimization of HPV DNA detection in urine by improving collection, storage, and extraction, Eur. J. Clin. Microbiol. Infect. Dis. 33 (11) (Nov 2014) 2005–2014, doi:10.1007/s10096-014-2147-2.
[10] H. zur Hausen, Condylomata acuminata and human genital cancer, Cancer Res. 36 (2 pt 2) (1976) 794.
[11] G. Koliopoulos, V.N. Nyaga, N. Santesso, et al., Cytology versus HPV testing for cervical cancer screening in the general population, Cochrane Database Syst. Rev. 8 (8) (2017) CD008587, doi:10.1002/14651858.CD008587.pub2.
[12] G. Ronco, J. Dillner, K.M. Elfstrom, et al., Efficacy of HPV-based screening for prevention of invasive cervical cancer: follow-up of four European randomised controlled trials, Lancet 383 (9916) (2014) 524–532, doi:10.1016/S0140-6736(13)62218-7.
[13] M. Arbyn, G. Ronco, A. Anttila, et al., Evidence regarding human papillomavirus testing in secondary prevention of cervical cancer, Vaccine 30 (Suppl. 5) (2012 Nov 20) F88–F99, doi:10.1016/j.vaccine.2012.06.095.
[14] M. Arbyn, E. Peeters, I. Benoy, et al., VALHUDES: a protocol for validation of human papillomavirus assays and collection devices for HPV testing on self-samples and urine samples, J. Clin. Virol. 107 (Oct 2018) 52–56, doi:10.1016/j.jcv.2018.08.006.
[15] M. Arbyn, F. Verdoodt, P.J. Snijders, et al., Accuracy of human papillomavirus testing on self-collected versus clinician-collected samples: a meta-analysis, Lancet Oncol. 15 (2) (2014) 172–183, doi:10.1016/S1470-2045(13)70570-9.
[16] S. Van Keer, E. Peeters, D. Vanden Broeck, et al., Clinical and analytical evaluation of the RealTime high risk HPV assay in Colli-pee collected first-void urine using the VALHUDES protocol, Gynecol. Oncol. 162 (3) (Sep 2021) 575–583, doi:10.1016/j.ygyno.2021.06.010.
[17] A. Latsuzbaia, D. Vanden Broeck, S. Van Keer, et al., Clinical performance of the RealTime high risk HPV assay on self-collected vaginal samples within the VALHUDES framework, Microbiol. Spectr. 10 (5) (Oct 26 2022), e0163122, doi:10.1128/spectrum.01631-22.
[18] A. Latsuzbaia, S. Van Keer, D. Vanden Broeck, et al., Clinical accuracy of Alinity m HR HPV assay on self- versus clinician-taken samples using the VALHUDES protocol, J. Mol. Diagn. 25 (12) (Dec 2023) 957–966, doi:10.1016/j.jmoldx.2023.09.008.
[19] A. Latsuzbaia, D. Vanden Broeck, S. Van Keer, et al., Validation of BD Onclarity HPV assay on vaginal self-samples versus cervical samples using the VALHUDES protocol, Cancer Epidemiol. Biomarkers Prev. 31 (12) (Dec 5 2022) 2177–2184, doi:10.1158/1055-9965.EPI-22-0757.
[20] S. Van Keer, A. Latsuzbaia, D. Vanden Broeck, et al., Analytical and clinical performance of extended HPV genotyping with BD Onclarity HPV assay in home-collected first-void urine: a diagnostic test accuracy study, J. Clin. Virol. 155 (Oct 2022), 105271, doi:10.1016/j.jcv.2022.105271.
[21] A. Latsuzbaia, D. Vanden Broeck, S. Van Keer, et al., Comparison of the clinical accuracy of Xpert HPV assay on vaginal self-samples and cervical clinician-taken samples within the VALHUDES framework, J. Mol. Diagn. 25 (9) (Sep 2023) 702–708, doi:10.1016/j.jmoldx.2023.06.004.
[22] A. Latsuzbaia, S. Van Keer, D.V. Broeck, et al., Accuracy of Liferiver HarmoniaHPV and VenusHPV assays on urine and vaginal self-samples, J. Med. Virol. 97 (3) (2025), e70273, doi:10.1002/jmv.70273.
[23] S. Van Keer, A. Latsuzbaia, D.V. Broeck, et al., Equivalent clinical accuracy of human papillomavirus DNA testing using cobas 4800 and 6800 human papillomavirus systems in paired urine and cervical samples, J. Mol. Diagn. (2025), doi:10.1016/j.jmoldx.2025.02.004.
[24] J. Viti, M. Poljak, A. Ostrbenk, et al., Validation of EUROArray HPV test using the VALGENT framework, J. Clin. Virol. 108 (Nov 2018) 38–42, doi:10.1016/j.jcv.2018.09.005.
[25] M. Arbyn, M. Simon, E. Peeters, et al., 2020 list of human papillomavirus assays suitable for primary cervical cancer screening, Clin. Microbiol. Infect. 27 (8) (Aug 2021) 1083–1095, doi:10.1016/j.cmi.2021.04.031.
[26] BE 71030031000, Biobank Antwerp [BB190007] BBMR-ERIC, Belgian, [BIORESOURCE] 2025.
[27] B. Serrano, R. Ibanez, C. Robles, P. Peremiquel-Trillas, S. de Sanjose, L. Bruni, Worldwide use of HPV self-sampling for cervical cancer screening, Prev. Med. 154 (Jan 2022), 106900, doi:10.1016/j.ypmed.2021.106900.
[28] L. Cadman, C. Reuter, M. Jitlal, et al., A randomized comparison of different vaginal self-sampling devices and urine for human papillomavirus testing-predictors 5.1, Cancer Epidemiol. Biomarkers Prev. 30 (4) (Apr 2021) 661–668, doi:10.1158/1055-9965.EPI-20-1226.
[29] S. Jordaens, K. Zwaenepoel, W. Tjalma, et al., Urine biomarkers in cancer detection: a systematic review of preanalytical parameters and applied methods, Int. J. Cancer 152 (10) (May 15 2023) 2186–2205, doi:10.1002/ijc.34434.
[30] M. Poljak, K. Cuschieri, L. Alemany, A. Vorsters, Testing for human papillomaviruses in urine, blood, and oral specimens: an update for the laboratory, J. Clin. Microbiol. 61 (8) (Aug 23 2023), e0140322, doi:10.1128/jcm.01403-22.
[31] M. Arbyn, S. Costa, A. Latsuzbaia, et al., HPV-based cervical cancer screening on self-samples in the netherlands: challenges to reach women and test performance questions, Cancer Epidemiol. Biomarkers Prev. 32 (2) (Feb 6 2023) 159–163, doi:10.1158/1055-9965.EPI-22-1041.
[32] A. Latsuzbaia, M. Martinelli, C. Giubbi, et al., Clinical accuracy of OncoPredict HPV quantitative typing (QT) assay on self-samples, J. Clin. Virol. 175 (Dec 2024), 105737, doi:10.1016/j.jcv.2024.105737.
[33] E. Rohner, L. Rahangdale, B. Sanusi, et al., Test accuracy of human papillomavirus in urine for detection of cervical intraepithelial neoplasia, J. Clin. Microbiol. 58 (3) (Feb 24 2020)doi:10.1128/JCM.01443-19.
[34] World Health Organization, Human papillomavirus vaccines: WHO position paper (2022 update), Wkly Epidemiol. Rec. 97 (50) (2022) 645–672 16–12-2022.
[35] IARC, IARC Handbooks of Cancer Prevention: Cervical Cancer Screening, 18, 2022.
[36] F. Wei, D. Georges, I. Man, I. Baussano, G.M. Clifford, Causal attribution of human papillomavirus genotypes to invasive cervical cancer worldwide: a systematic analysis of the global literature, Lancet 404 (10451) (Aug 3 2024) 435–444, doi:10.1016/S0140-6736(24)01097-3.
[37] A.M. Cornall, M. Poljak, S.M. Garland, et al., Anyplex II HPV28 detection and Anyplex II HPV HR detection assays are highly concordant with other commercial assays for detection of high-risk HPV genotypes in women with high grade cervical abnormalities, Eur. J. Clin. Microbiol. Infect. Dis. 36 (3) (Mar 2017) 545–551, doi:10.1007/s10096-016-2831-5.
[38] A.M. Cornall, M. Poljak, S.M. Garland, et al., EUROarray human papillomavirus (HPV) assay is highly concordant with other commercial assays for detection of high-risk HPV genotypes in women with high grade cervical abnormalities,

Eur. J. Clin. Microbiol. Infect. Dis. 35 (6) (Jun 2016) 1033–1036, doi:10.1007/s10096-016-2634-8.
[39] A. Latsuzbaia, J. Tapp, T. Nguyen, et al., Analytical performance evaluation of Anyplex II HPV28 and Euroarray HPV for genotyping of cervical samples, Diagn. Microbiol. Infect. Dis. 85 (3) (Jul 2016) 318–322, doi:10.1016/j.diagmicrobio.2016.04.011.
[40] A.R. Brentnall, K. Cuschieri, A. Sargent, J. Berkhof, M. Rebolj, Staged design recommendations for validating relative sensitivity of self-sample human papillomavirus tests for cervical screening, J. Clin. Epidemiol. 166 (Feb 2024), 111227, doi:10.1016/j.jclinepi.2023.111227.