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High-risk human papillomavirus testing in first-void urine as a novel and non-invasive cervical cancer screening modality—a Danish diagnostic test accuracy study

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Abstract

Background First-void urine (FVU) collection for high-risk human papillomavirus (hrHPV) testing has game-changing potential to improve cervical cancer prevention among under-screened women who remain unreached by clinician-based cervical cancer screening and vaginal self-sampling. Yet, the wide variation in the clinical accuracy of hrHPV testing in urine for detecting high-grade cervical intraepithelial neoplasia (CIN2 + /CIN3 +) across studies and clinical settings highlights the importance of local piloting and validation. This study determined the relative clinical accuracy of hrHPV testing in FVU versus clinician-collected cervical samples to detect CIN2 + /CIN3 + in a Danish referral population.

Methods In a diagnostic test accuracy study, paired FVU (10 mL Colli-Pee device; index test) and cervical samples (Cervex Combi brush; comparator test) were obtained from 325 women aged 23–64 years (median age 36.0 years (IQR 29–46) who were either referred for colposcopy and biopsy taking or a cervical excision (reference test; available for all participants). Samples were tested using Allplex HR HPV DNA extended genotyping assay. Same absolute cut-off for hrHPV positivity applied for cervical samples was used for FVU. Of the 325 women, 145 (44.6%), 180 (55.4%), and 138 (42.5%) were diagnosed with < CIN2, CIN2 +, and CIN3 +, respectively.

Results Sensitivity to detect CIN2 + (ratio 0.97, 95% CI 0.92–1.02, $p_{MCN} = 0.33$) and CIN3 + (ratio 0.95, 95% CI 0.90–1.00, $p_{MCN} = 0.09$) using hrHPV testing in FVU samples was not significantly different to hrHPV testing in cervical samples, whereas specificity for < CIN2 (ratio 0.67, 95% CI 0.46–0.96, $p_{MCN} = 0.04$) was significantly lower in FVU than on cervical samples. Moderate to excellent hrHPV test agreements between paired samples were demonstrated (Cohen's kappa = 0.44 to 0.88).

Conclusions This is the first study proving similar CIN2 + /CIN3 + sensitivity for FVU-hrHPV testing using the 10-mL Colli-Pee device and Allplex HR HPV assay compared to testing in cervical samples. From an implementation perspective, further research is needed to gather additional clinical accuracy and acceptability data on hrHPV testing of FVU-device collection in under-screened populations to support its broader integration into screening programmes.

Trial registration Clinicaltrials.gov: NCT05065853.

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Keywords Cervical cancer screening, Mass screening, HrHPV DNA testing, Urinary hrHPV testing, Uterine cervical dysplasia, Sensitivity and specificity, Early detection of cancer/methods

Background

With superior sensitivity to detect high-grade cervical intraepithelial neoplasia (CIN grades 2 and 3) and cancer and greater protection against cervical cancer, high-risk human papillomavirus (hrHPV) testing is replacing cytology as the primary cervical cancer screening modality in several countries [1, 2]. Unlike cytology, hrHPV testing can be performed on self-collected vaginal or urine samples [3]. Several countries including Denmark offer vaginal self-sampling for hrHPV testing to reach under-screened women who have the highest risk for developing cervical cancer due to the absence of early detection and effective treatment [4–6].

Still, recent data indicate that up to one-third of the women offered vaginal hrHPV self-sampling remain unscreened [7]. To engage women who are currently unreached by clinician-based cervical cancer screening and vaginal self-sampling, urinary hrHPV testing is fast becoming an alternative non-invasive screening option [8–10]. Although urine collection has proven highly acceptable and effective in engaging non-participants [11–13], the existing data regarding the sensitivity of urinary hrHPV testing to detect CIN grade 2 and worse (CIN2 +) and grade 3 and worse (CIN3 +) are inconsistent and sparse [14–23]. Inconsistencies observed between studies may be explained by non-standardized urine collection methods, the use of random or mid-stream-void urine instead of first-void urine (FVU), suboptimal processing conditions, various hrHPV tests [24], and differences in reference standard for disease verification [17, 18, 25–27]. Especially, collection of FVU is essential, as this initial flow of urine contains washed-away exfoliated cervical cells and debris containing significantly more human and hrHPV DNA than subsequent urine fractions [9]. Recently, two Belgian VALHUDES studies reported similar sensitivity of hrHPV testing in home-collected FVU versus clinician-collected cervical samples to detect CIN2 +/CIN3 + using a combination of a standardized FVU collection device and clinically validated PCR-based hrHPV DNA assays within a referral population [17, 18]. Herein, the histological reference test was based on colposcopy-directed biopsy if clinically indicated. Women with a normal colposcopy impression did not have biopsy collected (49%) and were grouped as disease-negative [17, 18]. This circumstance could potentially have influenced the overall accuracy assessments, especially because multiple studies have demonstrated an increase in the CIN2 + detection rate with increasing number of biopsies collected, even when colposcopy is normal [26, 28]. Thus, these results of clinical non-inferiority of FVU-hrHPV testing need to be confirmed in other studies using different disease verification strategies [28]. Therefore, this study focused on exploring the relative clinical accuracy of hrHPV testing in FVU collected with a standardized collection device, compared to clinician-collected cervical samples to detect high-grade cervical neoplasia (CIN2 +/CIN3 +) in a Danish referral population including women undergoing multiple biopsies regardless of colposcopy findings or cervical excision.

Methods

Setting

Danish women aged 23–64 years are invited for cervical cancer screening by liquid-based cervical cytology at their general practitioner. All screening, diagnostic, and treatment work-up are free-of-charge [29]. During this study, women aged 23–29 underwent cytology-based screening, whereas women aged 30–59 underwent cytology-based screening if born on even dates and hrHPV-based screening with cytology-triage if born on odd dates [30]. Women aged 60–64 had an hrHPV-DNA exit test [29] with cytology triage if hrHPV-positive. This current study took place in the Central Denmark Region, where all cervical cytology samples are routinely handled and analysed by the Dept. of Pathology, Randers Regional Hospital.

Study design and population

This study was designed as a diagnostic test accuracy study using a paired design following the VALHUDES validation framework [31] and STARD guidelines [32] and was conducted within a referral population between October 2021 and February 2023. Women provided an index test (FVU) and a comparator test (clinician-collected cervical sample) which were tested for hrHPV DNA. All women underwent a histological reference test (colposcopy-directed or random cervical biopsies or cervical excision) for disease verification. Paired samples were consecutively collected from women aged 23–64 years scheduled for a (1) colposcopy due to abnormal cervical cancer screening result or post-coital bleeding or (2) loop electrosurgical excision procedure (LEEP) at colposcopy clinics at Randers, Horsens, and Gødstrup Hospitals, Central Denmark Region. Attached to the appointment letter, the women received written

information about the study and were recommended if possible not to extensively wash their genitals on the day of the appointment and avoid urination 1 to 2 hours prior to the FVU collection at the clinic [9]. At enrolment, all included women provided information about their compliance with these recommendations. Exclusion criteria were known pregnancy at colposcopy/LEEP, menstruation, having given birth within the previous 3 months, and not understanding the Danish study material.

Sample collection

At the clinic and following written informed consent, participants were given oral and picture-based information on how to collect the FVU sample using the 10-mL Colli-Pee device (Colli-Pee, Novosanis, Wijnegem, Belgium). The Colli-Pee device collects approximately 7 mL of the FVU, whilst immediately mixing the urine with a DNA preservative (UCM, Novosanis, Belgium), reaching a total volume of 10 mL. Women also collected a vaginal self-sample at the clinic and answered a questionnaire about acceptability and preferences of the different sampling methods. These results will be reported in separate papers. Before colposcopy and LEEP, the clinician collected a cervical sample using a Cervex-Brush Combi (Rovers Medical Devices, Oss, The Nederlands) which was subsequently rinsed in 20-mL Thinprep PreservCyt medium (Hologic, Inc., Bedford, Massachusetts, USA). Samples were stored at room temperature at the colposcopy clinics until shipment to the laboratory at the Department of Pathology, Randers Regional Hospital.

Clinical procedures and histological outcomes

Women were managed clinically according to national guidelines [33]. For women referred for colposcopy, it is recommended to collect four cervical punch biopsies regardless of the hrHPV/cytology results, colposcopy findings, and risk factors. LEEP is recommended for women diagnosed with CIN grade 3 (CIN3) and adenocarcinoma in situ (AIS), whilst women with CIN2 may be referred for LEEP if they are outside the reproductive age or have no fertility desire. Pending on risk factors, screening results, and patient preferences, LEEP is also recommended as part of the diagnostic work-up for women without a fully visible transformation zone and/or when sufficient sampling is not possible. Histological results were reported using the CIN classification [34] and grouped as <CIN2 (normal including inflammation and non-specific reactive features, and CIN1), CIN2 + (CIN2, CIN3, AIS and cancer) and CIN3 + (CIN3, AIS, and cancer). Pathologists were blinded to the hrHPV results of the index and comparator samples. Histological results were retrieved using the nationwide Danish Pathology Databank [7], whereas hrHPV results of the study samples were delivered by the Department of Pathology, Randers Regional Hospital.

Sample processing, storage, and hrHPV analysis

After sample collection, the FVU was transported at room temperature for maximum 2 hours before arrival at the laboratory, whereas the cervical sample was transported at room temperature for 1 to 6 days (median 2 days) before arrival at the laboratory. Upon arrival in the laboratory, FVU samples and the ThinPrep PreservCyt vial with the cervical cells were vortexed for 20 s and afterwards storted at 4 °C for maximum 2 days before being aliquoted into secondary tubes and afterwards stored at −80 °C until hrHPV testing. Before the day of hrHPV analysis, the FVU aliquot (2 mL) and the cervical aliquot (0.6 mL) were thawed overnight at 4 °C (after storage at −80 °C for 9 to 24 months). On the day of hrHPV testing, a 2 mL volume of the FVU was centrifugated at 4000 × *g* for 20 min and 1.5 mL of the supernatant was removed and the pellet re-suspended in the remaining volume (0.5 mL) and transferred to an empty 1.5-mL tube. The cervical aliquot (0.6 mL) was vortexed for 20 s and transferred to an empty 1.5mL tube. hrHPV DNA testing was performed using real-time PCR-based Allplex HPV HR detection assay (Seegene, Korea) targeting the HPV L1 region [35]. It detects 14 individual hrHPV types: hrHPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 and generates individual quantitative readouts (Cycle Threshold, Ct) for each genotype [35]. DNA extraction was performed using STARMag Universal Cartridge kit on the STARLET IVD platform (Hamilton, USA) followed by PCR amplification on a CFX96™ real-time thermocycler (Bio-Rad, USA) according to the manufacturer's instructions. For DNA extraction, input volume was 200 µL for FVU and cervical samples with elution volumes of 60 µL and 100 µL for FVU and cervical samples, respectively. Data analysis of the genotyping results was interpreted and automatically analysed on the Seegene Viewer software. As no cut-off for hrHPV positivity in FVU has been established by the manufacturers, we used the manufacturer's predefined absolute threshold for hrHPV positivity in cervical samples (all hrHPV genotypes: Ct ≤43), also for FVU. When performing our HPV analyses in 2023, the subsequently published genotype-specific clinical thresholds for hrHPV positivity in cervical samples [35] had not yet been included in the package insert for the Allplex assay. For FVU, collected by the 10 or 20 mL Colli-Pee device or a urine cup, no clinical thresholds have been established for the Allplex assay to date. This was the reason for applying the absolute rather than the clinical thresholds for both cervical and FVU samples in our main analyses. Samples with

an invalid test result (no β-globin gene detected) were retested once and the second result was considered definitive. Each run also included three positive controls. The cytotechnicians performing the hrHPV testing were blinded to all study outcomes except the woman's age.

Statistical analyses and sample size

We evaluated the hrHPV DNA positivity across individual disease categories and sample types by cross-tabulating histology and hrHPV positivity for each sample type using the absolute thresholds for hrHPV positivity. We estimated the relative and absolute clinical accuracy (sensitivity for CIN2 + and CIN3 +, and specificity for < CIN2) with exact 95% confidence intervals (95% CIs) of hrHPV testing in FVU and cervical samples. Tests for differences in hrHPV positivity, sensitivity, and specificity in paired FVU vs cervical samples were performed using the McNemar's (McN) test. For continuous data, the median and interquartile ranges (IQR) were calculated. Differences in proportions for unpaired comparisons were tested with two-sample test. Overall percentage agreement and Cohen's Kappa (κ) test concordance for any hrHPV and specific hrHPV genotypes were assessed between the paired samples. Cohen's κ was grouped into: "poor" ($\kappa \leq 0.20$), "fair" ($0.21 \leq \kappa \leq 0.40$), "moderate" ($0.41 \leq \kappa \leq 0.60$), "good" ($0.61 \leq \kappa \leq 0.80$), or "excellent" ($\kappa \geq 0.81$) [36]. To address small numbers within specific genotype strata, the genotypes were grouped into three groups based on their carcinogenicity for CIN2 +/CIN3 + as reported by Bonde et al. [37]: (1) highest carcinogenicity (hrHPV16/18), (2) intermediate carcinogenicity (hrHPV31,33,45,51,52,58), and (3) lowest carcinogenicity (hrHPV35,39,56,59,66,68). Compliance to avoid urination and thoroughly intimate wash before FVU collection were grouped as (1) non-compliant: both urination and intimate wash was reported, (2) partly compliant: urination or intimate wash was reported, and (3) fully compliant: no urination or intimate wash was reported. In sensitivity analyses, we evaluated if the test accuracy changed when we restricted the study population to women aged ≥ 30 years and women undergoing colposcopy due to abnormal screening results (i.e., excluding women referred for colposcopy due to post-coital bleeding symptoms and those undergoing LEEP). A supplementary analysis was performed to explore the effect on test accuracy between sample types using the absolute (Ct ≤ 43) and later published clinical genotype-specific thresholds for hrHPV positivity in cervical samples [35]. Herein, hrHPV positivity was defined as Ct ≤ 40 for hrHPV16/18, Ct ≤ 37 for hrHPV31/33/45/52/58, and Ct ≤ 35 for hrHPV35/39/51/56/59/66/68 [35]. Data was stored and entered in REDCap [38]. The statistical analyses were conducted using STATA version 18.

The sample size calculation focused on estimating the sensitivity of FVU-hrHPV testing for CIN2 + with sufficient precision. The study was scaled to estimate a 95% CI with a width of ± 5%. Thus, assuming 88% sensitivity and 90% specificity of urinary hrHPV testing [19] for CIN2 +, 169 CIN2 + cases and 152 < CIN2 cases totaling at least 321 women had to be included [39].

Ethical approval

The project was listed in the record of processing activities for research projects in the Central Denmark Region (j.no. 1–16-02–313-21) and approved by the Ethics Committee in the Central Denmark Region (j. no: 1–10-72–246-21). All participants provided written informed consent.

Results

Study population

A total of 493 women were eligible for inclusion whereof 369 women (74.8%) consented and were enrolled in the study (Fig. 1). After study exclusions (8.9%), 325 women (median age 36.0 years (IQR 29–46) with valid hrHPV results for the paired samples and histological results were included for analysis (Fig. 1 and Table 1). Most women underwent colposcopy (60.6%, $n = 197/325$) and were in general referred for colposcopy or LEEP based on abnormal cytology (59.7%, $n = 194/325$). About half of the women (56.4%, $n = 182/325$) reported to have been fully compliant with the recommendations to avoid urination in 1–2 hours before FVU collection and thoroughly intimate wash the day of study enrollment. Among partly compliant women (31.3%, $n = 101/325$), most had avoided urination but reported intimate wash (62%, $n = 63/101$) (Table 1).

hrHPV positivity by sampling method and histological results

Of the 325 included women, a total of 145 women (44.6%) were diagnosed with < CIN2 and 180 women (55.4%) were diagnosed with CIN2 + including two squamous cell carcinomas (Table 2). The hrHPV positivity was similar for FVU and cervical samples (88.0%, 95% CI 84.0–91.3% vs 86.8%, 95% CI 82.6–90.3%, $p = 0.63$). Across sampling methods, the hrHPV positivity increased with the severity of histology from normal to CIN3/AIS for FVU (83.3 to 91.7%, respectively, $p = 0.48$) and cervical samples (67.9 to 100%, respectively, $p < 0.01$) (Table 2). Both sampling methods were hrHPV-positive in the two cancer cases (Table 2).

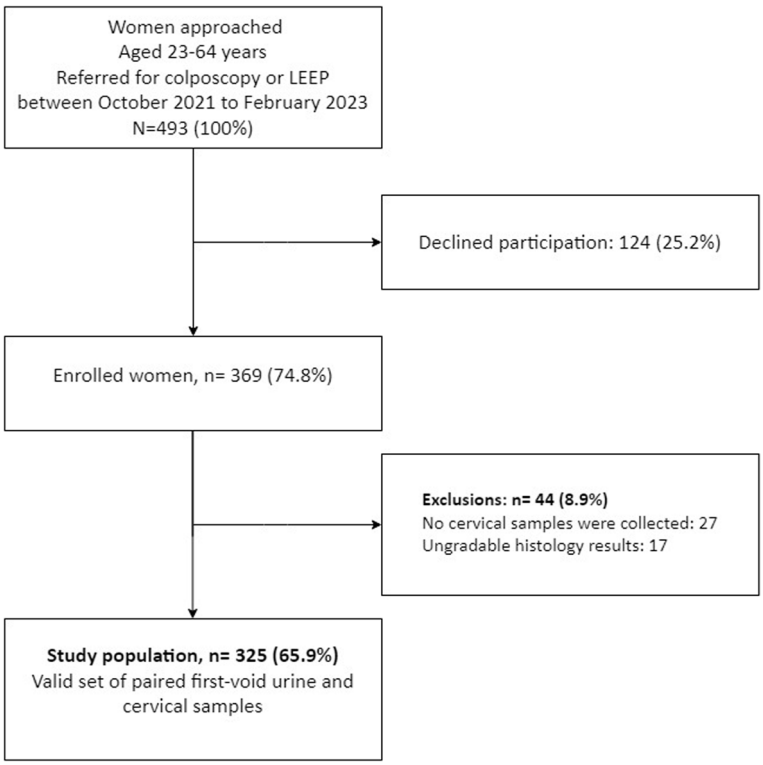

Fig. 1 Flowchart of the study population LEEP: Loop Electrosurgical Excision Procedure. Exclusions: No cervical samples were collected because the clinician forgot to obtain the sample before the colposcopy or LEEP procedure

Table 1 Characteristics of study population ($n = 325$)

Median age at time of inclusion, years (IQR)	**36 (29–46)**
Age groups, *n* (%)	
23–29	87 (26.7)
30–36	78 (24.0)
37–43	63 (19.4)
44–64	97 (29.9)
Referred for colposcopy or LEEP, *n* (%)	
Colposcopy	197 (60.6)
LEEP	128 (39.4)
Referral test, ***n*** (%)	
Primary HPV with reflex cytology	131 (40.3)
Primary cytology with reflex HPV test	194 (59.7)
Compliance to avoid urination and intimate wash before FVU collection*, *n* (%)	
Non-compliant	40 (12.4)
Fully compliant	182 (56.4)
Partly compliant	101 (31.3)
Don't know	2 (0.6)

LEEP Loop Electrosurgical Excision Procedure, *FVU* First-void-urine, *IQR* interquartile range, *n* number

* Non-compliant: Urination and intimate wash was reported; Fully compliant: No urination or intimate wash was reported; Partly compliant: Urination or intimate wash was reported

Table 2 hrHPV positivity in relation to histological results in first-void urine and cervical samples

		hrHPV positivity, *n* (%)**	
Histology results	***n* (%)***	**First-void urine**	**Cervical samples**
Normal	78 (24.0)	65 (83.3)	53 (67.9)
CIN1	67 (20.6)	58 (87.0)	59 (88.0)
CIN2	42 (13.0)	38 (90.5)	39 (92.9)
CIN3	124 (38.1)	112 (90.3)	117 (94.3)
AIS	12 (3.7)	11 (91.7)	12 (100)
Carcinoma	2 (0.6)	2 (100)	2 (100)
Total	325 (100)	286 (88.0)	282 (86.8)

CIN cervical intraepithelial neoplasia, grades 1 to 3, *AIS* adenocarcinoma in situ

hrHPV positivity: HPV16,18,31,33,35,39,45,51,52,56,58,59,66 and 68

*column percentages

**row percentages

Clinical accuracy

FVU and cervical hrHPV testing correctly identified 91.0% ($n = 163/180$) and 94.4% ($n = 170/180$) of the CIN2 + cases, respectively (Table 3). Of the 138 CIN3 + cases, 125 FVU samples were hrHPV positive, in comparison to 131 cervical samples corresponding to absolute sensitivities of respectively 90.6% ($n = 125/138$) and 94.9% ($n = 131/138$). Out of 145 < CIN2 cases, 22 were hrHPV

Table 3 Comparison of absolute and relative accuracy of hrHPV testing in first void urine as compared to cervical samples

		CIN2 + sensitivity ($n = 180$)			CIN3 + sensitivity ($n = 138$)				< CIN2 specificity ($n = 145$)			
	n^a	Absolute % (95% CI)	Relative (95% CI)	P_{MCN}	n^b	Absolute % (95% CI)	Relative (95% CI)	P_{MCN}	n^c	Absolute % (95% CI)	Relative (95% CI)	P_{MCN}
Total study population ($n = 325$)												
Cervical	170/180	94.4 (90.0–97.3)	1		131/138	94.9 (89.8–98.0)	1		33/145	22.8 (16.2–30.5)	1	
FVU	163/180	91.0 (85.3–94.4)	0.97 (0.92–1.02)	0.33	125/138	90.6 (84.4–94.9)	0.95 (0.90–1.00)	0.09	22/145	15.2 (9.7–22.1)	0.67 (0.46–0.96)	0.04
Women aged ≥ 30 years ($n = 238$)												
Cervical	133/141	94.3 (89.1–97.5)	1		102/108	94.4 (88.3–97.9)	1		22/97	22.7 (14.8–32.3)	1	
FVU	128/141	90.8 (84.7–95.0)	0.96 (0.91–1.02)	0.27	98/108	90.7 (83.7–95.5)	0.94 (0.88–1.00)	0.11	17/97	17.5 (10.6–26.6)	0.77 (0.50–1.20)	0.36

hrHPV positivity: HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68

P_{MCN}, *P*-value for McNemar's test

FVU first void urine, *CIN* cervical intraepithelial neoplasia; CIN2 + CIN2, CIN3/AIS (adenocarcinoma in situ) and cancer, *CIN3* + CIN3/AIS and cancer, < *CIN2* Normal and CIN1

[a] True positive/total CIN2 + cases

[b] True positive/total CIN3 + cases

[c] True negative/total < CIN2 cases

negative in FVU and 33 in cervical samples with corresponding specificity of 15.2% (n = 22/145) and 22.8% (n = 33/145), respectively. We found no significant differences in median age (37.0 vs 36.0 years, p = 0.73) between 17 false negative FVU cases and the 163 true positive FVU cases and between the 10 false negative cervical sample cases and the 170 true positive cervical sample cases (35.5 vs 36.0, p = 0.94) (data not tabulated). Four CIN3 cases were tested hrHPV negative on cervical samples only, six CIN2 + (three CIN2 and three CIN3) were tested hrHPV negative in both methods, whilst 11 CIN2 + cases (one CIN2, 9 CIN3, and one AIS) were tested hrHPV negative in FVU only. Of the 11 CIN2 + cases, 55% of the women (n = 6/11) reported to have been partly compliant by avoiding urination, but reported intimate wash before FVU collection, 36% (n = 4/11) have been fully compliant, and 9% (n = 1/11) were not compliant (data not tabulated). Compared to hrHPV testing in cervical samples, hrHPV testing in FVU samples was found similarly sensitive for CIN2 + (ratio 0.97, 95% CI 0.92–1.02, p = 0.33) and CIN3 + (ratio 0.95, 95% CI 0.90–1.00, p = 0.09) but significantly less specific to exclude underlying < CIN2 (ratio 0.67, 95% CI 0.46–0.96, p = 0.04) (Table 3). When restricting the analyses to women aged 30 and above, the sensitivity of hrHPV testing in FVU as compared to cervical samples for CIN2 + (ratio 0.96, 95% CI 0.91–1.02, p = 0.27) and CIN3 + (ratio 0.94, 95% CI 0.88–1.00, p = 0.11) was similar to those of the main analysis but without significantly differences in specificity (ratio 0.77, 95% CI 0.50–1.20, p = 0.36) (Table 3). Among women referred for colposcopy due to abnormal screening results, the relative sensitivity of FVU-hrHPV testing was equivalent to cervical sampling for CIN2 + (ratio 1.00, 95% CI 0.94–1.06, p = 1.00) and CIN3 + (ratio 1.00, 95% CI 0.92–1.09, p = 1.00) endpoints with significantly lower specificity (ratio 0.56, 95% CI 0.35–0.88, p = 0.02) (Additional file 1: Table. S1). Using the clinical thresholds for hrHPV positivity established for cervical samples in both samples, the relative clinical sensitivity of FVU was significantly lower for CIN2 + (ratio 0.91; 95% CI 0.85–0.97, p = 0.01) and CIN3 + (ratio 0.91; 95% CI 0.85–0.98, p = 0.03), with no significant difference in specificity for < CIN2 (ratio 0.91, 95% CI 0.69–1.20, p = 0.63) (Additional file 1: Table. S2). However, when maintaining the absolute threshold for FVU and using the clinical threshold for cervical samples, the sensitivities for CIN2 + (ratio 0.98; 95% CI 0.93–1.03; p = 0.48) and CIN3 + (ratio 0.98; 95% CI 0.92–1.04, p = 0.63) were virtually equal between FVU and cervical samples, though specificity for < CIN2 was significantly lower (ratio 0.48; 95% CI 0.33–0.68; p = 0.01) (Additional file 1: Table. S2).

hrHPV genotype agreement

Multiple hrHPV-type infections were more common in FVU (42.3%, n = 121/286) than in the cervical samples (32.3%, n = 91/282, p < 0.01) (data not tabulated). The same genotype(s) were detected in 63.3% (n = 167/264) of the paired hrHPV-positive FVU and cervical samples, whilst at least one identical genotype was found in 35% of the paired samples (n = 92/264) with the remaining 2% of the paired samples without any agreement (5/264) (data not tabulated). The overall percent agreement for any hrHPV detection between paired samples was 87.7% (Table 4). Between FVU and cervical samples, Cohen's

Table 4 Comparison of test concordance and hrHPV genotype test concordance between first-void urine and cervical samples (n = 325)

hrHPV results	Sampling methods compared	No of samples				Overall % agreement (95% CI)	Cohen's kappa (95% CI)
		+/+	−/−	+/−	−/+		
Any hrHPV[a] (14 types)	FVU/cervical	264	21	22	18	87.7 (83.6–91.1)	0.44 (0.30–0.59)
hrHPV16/18[b]	FVU/cervical	72	238	5	10	95.4 (92.5–97.4)	0.88 (0.81–0.94)
Genotypes with intermediate carcinogenicity[c]	FVU/cervical	156	130	24	15	88.0 (84.0–91.3)	0.76 (0.69–0.83)
Genotypes with lowest carcinogenicity[d]	FVU/cervical	89	185	43	8	84.3 (80.0–88.1)	0.66 (0.58–0.74)

FVU first void urine, +: hrHPV positive, −: hrHPV negative

The results indicated on either side of the slash corresponds to the sample type listed in the same position in the first column

Cohen's kappa: poor: 0.00–0.20: fair: 0.21–0.40; moderate: 0.41–0.60; good 0.61–0.80; and excellent: 0.81–1.00

[a] Any hrHPV: Positive for at least one genotype included in the Allplex hrHPV assay: HPV16, 18,31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68

[b] HPV16 and/or HPV18 including co-infections with genotypes included in the intermediate (hrHPV31,33,45,51,52,58) and lowest carcinogenicity (hrHPV35,39,56,59,66,68) groups

[c] hrHPV31,33,45,51,52, and/or 58 including co-infections with HPV16/18 and/or genotypes included in the group with lowest carcinogenicity (hrHPV35,39,56,59,66,68)

[d] hrHPV35,39,56,59,66 and/or 68 including co-infections with HPV16/18 and/or genotypes included in the group with intermediate carcinogenicity (hrHPV31,33,45,51,52,58)

Kappa test concordance was moderate for any hrHPV type (k= 0.44), excellent for hrHPV16/18 detection (k= 0.88), and good for genotypes found to have intermediate (k= 0.76) and lowest (k= 0.66) oncogenicity for CIN2 +/CIN3 + (Table 4).

Discussion

Main findings

To our knowledge, this study was the first to explore the clinical accuracy of FVU-hrHPV testing using the 10-mL ColliPee device to detect high-grade CIN lesions using the extended genotyping Allplex HR HPV detection assay. No significantly difference in clinical sensitivity for CIN2 + and CIN3 + was found for hrHPV testing in paired FVU and clinician-collected cervical samples, whereas FVU-hrHPV testing had significantly lower specificity for < CIN2. Among women aged 30 and above, similar sensitivities for CIN2 + and CIN3 + were found without significantly loss in specificity for < CIN2 as compared to the total study population.

Comparison with other studies and interpretation

We observed for FVU, a directionality of higher hrHPV positivity with increasing grade of neoplasia as seen in cervical samples [40] (Table 2) and a high level of genotype-specific agreement (63.3%), supporting the utility of FVU sampling for evaluating hrHPV-induced cervical disease. In our study, the hrHPV positivity rate in FVU (88.0%) were comparable to cervical samples (86.8%) and in agreement with the pooled hrHPV positivity in FVU of 80% reported in a meta-analysis by Pathak et al. [9]. However, our hrHPV positivity rate of 88.0% in FVU was significantly higher than the rates reported by Van Keer et al. using the combination of the 20-mL ColliPee device and the extended hrHPV genotyping Abbott RealTime High Risk HPV assay (61.5%) [18] and BD Onclarity assay [17] (60.3%) in a Belgian referral population. This difference was likely explained by the higher disease prevalence in our study as compared to the Belgian cohort (55.4% vs 17.8%, respectively) [17, 18]. FVU-hrHPV testing is only beneficial if it can detect women with CIN2 and especially CIN3 lesions as these are treatable screening endpoints [41]. Encouragingly, our data showed that FVU-hrHPV testing was similarly sensitive to detect CIN2 + and CIN3 + as compared to cervical samples. The results are in alignment with some [14, 16–18, 23] but not all studies [15, 19, 22, 42, 43] conducted in referral populations where clinically validated PCR-based assays were used for hrHPV DNA testing. Results of these existing studies showed absolute sensitivities for CIN2 + ranging from 50.8% [43] to 95% [14, 16, 23], CIN3 + ranging from 88.9% [18] to 100% [23], and relative sensitivities ranging from 0.87 [44] to 1.00 [17] for CIN2 + and 0.91 [18] to 0.98 [17] for CIN3 +. Both the absolute sensitivities for CIN2 + (91.0%) and CIN3 + (90.6%) and the relative sensitivities (CIN2 +: 0.97 and CIN3 +: 0.95) found in our study fall inside these ranges. Since the CIN2 diagnosis is a more ambiguous diagnosis than CIN3 [45], it is reassuring that our study showed similar absolute sensitivity of FVU-hrHPV testing for both CIN2 + and CIN3 +. Despite that we found no statistically significant difference between sampling methods, FVU was relatively 3 and 5% less sensitive to detect CIN2 + and CIN3 +, respectively. The differences were most evident in women with CIN3, where FVU failed to detect nine cases that were hrHPV positive in the paired cervical samples. Imperfect sampling could have affected the hrHPV result for these FVU samples, but none of these nine women reported in the questionnaire problems in collecting the FVU samples. In a spin-off study, we will explore whether lack of compliance to avoid urination and especially intimate washing before FVU collection could lower the cellularity and thus be a potential contributor to this finding. As pointed out by others [46], some loss of sensitivity may be tolerated in under-screened women who are currently unreached by both clinician-based screening and vaginal self-sampling. However, if FVU-hrHPV testing is to be more widely used among regularly screened women, it would require FVU to be equally sensitive as cervical sampling; otherwise, the preventive benefit would be gone. This said, the absolute sensitivity of FVU-hrHPV testing (91%) to detect CIN2 + found herein was higher than the sensitivity of cytology alone (range 50–70%) [1] which has worked as the standard screening modality for decades. Still, further research is warranted to study the suitability of FVU as test material for primary screening as well as the potential need for FVU-specific clinical thresholds for hrHPV positivity for assays that have been clinically validated for cervical cancer screening using cervical samples [47]. Our supplementary data supported this need, showing virtually equal clinical sensitivity between FVU and cervical samples when the absolute threshold was maintained for FVU and the clinical threshold was applied to cervical samples. In contrast, applying the clinical threshold to both sample types resulted in reduced sensitivity for FVU. From an implementation perspective, integrating FVU collection into existing HPV-based cervical cancer screening programmes, particularly those that already include vaginal self-sampling, appears highly feasible. This is especially true with the 10-mL Colli-Pee device which due to its smaller size and more compact design as compared to the 20-mL Colli-Pee FVU collection device is more suitable for postal delivery enabling convenience and facilitate home-based collection [47]. Additionally, the collection tube could be adapted for

high-throughput laboratory workflows, further supporting its feasibility for large-scale screening [47]. These circumstances formed the rationale for choosing the 10-mL collection device in our study. As in other studies [17, 18], our results revealed good to excellent test concordance between FVU and cervical samples for the genotypes found by Bonde et al. to have the highest (HPV16/18) and intermediate carcinogenicity for CIN2 +/CIN3 + [37]. This may indicate that the proven value of extended genotyping beyond hrHPV16/18 triage testing for guiding colposcopy referral of women tested hrHPV-screen-positive in cervical samples may also be valid for FVU-hrHPV testing [48]. In an upcoming study, we will further explore the diagnostic utility of DNA hypermethylation profiling [49, 50] in hrHPV-positive FVU samples to differentiate between normal/CIN1 and CIN2/CIN3.

Strengths and limitations

Strength of our study includes the standardized FVU collection procedure that is suitable for postal delivery. As our study was among the very first to use the 10-mL Colli-Pee FVU collection device, it is reassuring that it showed comparable test sensitivity to the 20-mL Colli-Pee device, which has been extensively studied in the VALHUDES studies [17, 18, 47]—although not in combination with the Allplex HR HPV DNA assay. Furthermore, this study included several times as many CIN2 +/CIN3 + cases as the previous comparative studies (range 6–108) [16] exploring the clinical sensitivity of FVU-hrHPV-testing. High sensitivity of the reference test to ascertain the true disease status was secured as women included had multiple cervical biopsies collected regardless of colposcopy findings or had a LEEP performed, thereby minimizing verification bias. The paired design ensured that the women served as their own control, minimizing the risk for confounding, whilst collection of the paired sample sets at the same visit and immediately before colposcopy or LEEP excluded the option that the hrHPV-infection had been acquired or cleared between samples. Limitations include that FVU was collected at the colposcopy clinics under relatively controlled clinical circumstances which is not representative of its intended use in a home-based setting. Criticism may arise regarding the participant enrollment from colposcopy clinics, as it may not reflect the under-screened population for which FVU collection is intended to offer a solution. Since specificity decreases with increasing hrHPV prevalence [51], the absolute specificity reported in our study was lower than what would be expected in a screening population. However, Arbyn et al.'s meta-analysis demonstrated that the relative clinical accuracy of hrHPV-testing in self-collected vs cervical samples is convertible between referral and screening populations [3], supporting the robustness of our study conclusions. Nevertheless, ongoing scientific discussions revolve around identifying the optimal study design and population to accurately estimate the relative sensitivity of self-sample HPV tests for cervical cancer screening [52]. Lastly, women aged 23–64 were enrolled in this study although hrHPV-based screening is typically recommended for women aged ≥ 30 years in most (not all) countries. However, our sensitivity analysis showed that the sensitivity estimates among women aged 30 and older was similar to those of the total study population with a benefit in specificity for < CIN2. The latter was likely explained by the lower prevalence of transient hrHPV infections in women aged 30 + as compared to younger women (23–29 years).

Conclusions

This study proved hrHPV testing in FVU, collected using a standardized collection device, to have similar relative sensitivity to detect CIN2 + and CIN3 + but significantly lower specificity compared to hrHPV testing on clinician-collected cervical samples. The comparison was made using an extended hrHPV genotyping assay in a referral population where the histology reference test was available for all participants. Going forward, generating further clinical accuracy and acceptability data on FVU-hrHPV testing in under-screened populations is warranted to substantiate its implementation in screening programmes.

Abbreviations

CI	Confidence interval
CIN	Cervical intraepithelial neoplasia
CIN2 +	Cervical intraepithelial neoplasia grade 2 or worse
CIN3 +	Cervical intraepithelial neoplasia grade 3 or worse
DNA	Deoxyribonucleic acid
FVU	First-void urine
hrHPV	High-risk human papillomavirus
LEEP	Loop electrosurgical excision procedure
McN	McNemar's test
PCR	Poly-chain reaction

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12916-025-04149-0.

Additional File 1: Table S1-S2. Table S1 [Comparison of absolute and relative accuracy of hrHPV testing in first void urine as compared to cervical samples for women referred for colposcopy due to abnormal screening results]. Table S2 [Comparison of absolute and relative accuracy of hrHPV testing in first void urine as compared to cervical samples using absolute and clinical hrHPV positivity threshold].

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Authors' contributions
MT: scientific PI and study coordinator and was responsible for conducting the study overall. MT, SVK, LWG, and AV conceived the original idea. PN: refinement of the technical details for sample handling, archiving of the samples, and performing all HPV testing. JSJ contributed with comments on the laboratory part of the study. LWG, PB, AH, CB, and KOB elaborated of procedures for enrollment, colposcopy, biopsy taking, and cervical excision. SVK and AX: elaboration of handling and HPV testing on urine. MT was the first author and drafted the first version of this article, which was subsequently further developed by all authors, who also reviewed and approved this version for submission.

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Data availability
The dataset used in this study contains personal information and is not publicly available. An anonymized dataset is available from the corresponding author upon reasonable request and with permissions from relevant Danish Authorities.

Declarations

Ethics approval and consent to participate
The project was listed in the record of processing activities for research projects in the Central Denmark Region (j.no. 1–16-02–313-21) and approved by the Ethics Committee in the Central Denmark Region (j. no: 1–10-72–246-21). All participants provided written informed consent.

Consent for publication
Not applicable.

Competing interests
Seegene sponsors the Allplex HR HPV assays for the study. According to the contract between Seegene and the University Research Clinic for Cancer Screening and Dept. of Pathology, Randers Regional Hospital, Seegene had no influence on the scientific process and no editorial rights pertaining to this manuscript. The authors retained the right to submit the manuscript. MT and JSJ have participated in other studies with HPV test kits sponsored by Roche. MT has received honoraria fee from Roche Diagnostics and AstraZeneca for lectures on HPV self-sampling and HPV triage-methods, respectively. SVK was supported by a junior postdoctoral fellowship of the Research Foundation – Flanders (grant no: 1240220 N). The University of Antwerp received payment for participation of SVK in an Advisory Board of Novosanis (Subsidiary of OraSure Technologies Inc, Wijnegem, Belgium). All funds are handled and managed by the University of Antwerp. LWG and AH: Have outside this project, received free-of-charge test kits from Roche Diagnostics and AH has received honoraria fee from Exeltis. PN, PB, KOB, and CB: No competing interests. AV: is co-founder of and former board member of Novosanis (Subsidiary of OraSure Technologies Inc, Wijnegem, Belgium), a spin-off company of the University of Antwerp, and was minority shareholder until January 2019.

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