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RESEARCH ARTICLE

Urine high-risk human papillomavirus testing as an alternative to routine cervical screening: A comparative diagnostic accuracy study of two urine collection devices using a randomised study design trial

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Abstract

Objective: To evaluate the sensitivity of human papillomavirus (HPV) tested urine to detect high-grade cervical precancer (cervical intraepithelial neoplasia grade 2+ [CIN2+]) using two urine collection devices.

Design: Randomised controlled trial.

Setting: St Mary's Hospital, Manchester, UK.

Population: Colposcopy attendees with abnormal cervical screening; a total of 480 participants were randomised. Matched urine and cervical samples were available for 235 and 230 participants using a first-void urine (FVU)-collection device and standard pot, respectively.

Methods: Urine was self-collected and mixed with preservative – randomised 1:1 to FVU-collection device (Novosanis Colli-pee® 10 mL with urine conservation medium [UCM]) or standard pot. Matched clinician-collected cervical samples were taken before colposcopy. HPV testing used Roche cobas® 8800. A questionnaire evaluated urine self-sampling acceptability.

Main outcome measures: The primary outcome measured sensitivity of HPV-tested urine (FVU-collection device and standard pot) for CIN2+ detection. Secondary outcomes compared HPV-tested cervical and urine samples for CIN2+ and evaluated the acceptability of urine self-sampling.

Results: Urine HPV test sensitivity for CIN2+ was higher with the FVU-collection device (90.3%, 95% CI 83.7%–94.9%, 112/124) than the standard pot (73.4%, 95% CI 64.7%–80.9%, 91/124, $p = 0.0005$). The relative sensitivity of FVU-device-collected urine was 0.92 (95% CI 0.87–0.97, $p_{McN} = 0.004$) compared with cervical, considering that all women were referred after a positive cervical HPV test. Urine-based sampling was acceptable to colposcopy attendees.

Conclusions: Testing of FVU-device-collected urine for HPV was superior to standard-pot-collected urine in colposcopy attendees and has promising sensitivity for CIN2+ detection. General population HPV testing of FVU-device-collected urine will establish its clinical performance and acceptability as an alternative to routine cervical screening.

KEYWORDS
cervical screening, clinical validation, Colli-pee®, diagnostic test accuracy, first-void urine, human papillomavirus, human papillomavirus testing, Roche cobas® 8800, self-sampling

1 | INTRODUCTION

Cervical screening has reduced cancer-specific mortality by approximately 70% in the UK.[1] Despite its success, uptake is currently 68.7% and falling.[2] Reasons for non-attendance include access,[3] embarrassment and the speculum examination.[4] Urine high-risk human papillomavirus (HPV) testing is an attractive option for cervical screening because it has the potential to eliminate these barriers. A systematic review and meta-analysis of 14 studies and 1443 women reported a pooled sensitivity of 77% for HPV detection in urine.[5] Subsequent studies have focused on optimising urine collection, processing and testing protocols.[6–12] First-void urine (FVU) is important for test accuracy as it flushes periurethral mucus containing HPV-infected cellular debris, if present, into the sample collector.[13] Most previous studies used urine collected using the standard pot with wide-ranging test accuracy results.[5] A specialised FVU-collection device is now available, which collects a standardised small volume of urine and enables immediate mixing with preservative. This FVU-collection device has negative cost and environmental implications compared with a standard pot, however, making studies confirming its clinical effectiveness imperative in the justification of its use.[14,15]

We hypothesised that (i) HPV-tested urine has high sensitivity for high-grade cervical precancer (cervical intraepithelial neoplasia grade 2+ [CIN2+]) detection relative to cervical samples and that (ii) its accuracy is not affected by type of collection device.

The Alternative CErvical Screening (ACES) Colposcopy study aimed to compare the sensitivity of HPV-tested matched urine and cervical samples for CIN2+ detection using two urine collection devices. A secondary objective was to explore the acceptability of urine sampling versus clinician-collected samples for cervical screening among colposcopy clinic attendees.

2 | METHODS

2.1 | Trial design and participants

This was a prospective, parallel group, two-arm, non-inferiority trial with a 1:1 allocation ratio to urine collection device, conducted at the colposcopy department at St Mary's Hospital, Manchester University National Health Service (NHS) Foundation Trust (MFT) between May 2021 and February 2022. The study was approved by the North-West Greater Manchester Research Ethics Committee (20/NW/0389) and registered as a clinical trial (ISRCTN13132810). Funding was provided through an NIHR Advanced Fellowship (Crosbie, NIHR300650).

Potential participants were identified from colposcopy clinic lists. Eligible individuals were between 24 and 70 years old and referred to colposcopy clinic with abnormal cervical screening results: all following a positive cervical HPV test, 88% had abnormal cytology (Table S1). Pregnancy was an exclusion criterion for the study. A Patient and Public Involvement and Engagement group co-designed all participant-facing materials for the study. All participants provided written, informed consent to take part.

2.2 | Randomisation and blinding

A total of 480 participants were randomised (1:1) between two urine collection devices: an FVU-collection device (Novosanis Colli-pee® 10 mL with urine conservation medium [UCM®][16]) and the standard pot. The block randomisation method used a secure web-based system to prevent predictability of allocation. Randomisation used the RedCap database and group allocation was revealed to the researcher once eligibility, consent and participation were confirmed. The laboratory staff responsible for HPV testing the samples were blinded to participant cervical screening and medical history. Withdrawn participants were replaced with new participants within the same urine collection device arm.

2.3 | Interventions

Participants were asked to not urinate for at least 1 hour before providing a self-collected urine sample, in the privacy of the clinic bathroom, using the urine collection device to which they were randomised. For both devices, participants were shown how to use the devices and asked to provide FVU.

The FVU-collection device allowed standardised volumetric collection of urine (approximately 6.6 mL) directly into a tube containing UCM (3.4 mL) allowing for immediate preservative mixing. In the laboratory, 2 mL of the sample was placed in an empty tube compatible with the Roche cobas® 8800 testing platform. To balance obtaining a small volume (to avoid sample over-dilution) with the difficulty of producing a small volume sample using the standard pot, participants were asked to provide a FVU sample that filled the pot to approximately two-thirds of its capacity (60 mL maximum capacity). Approximately 8 mL of standard pot urine was transferred within 5 minutes of urine collection into a cobas® polymerase chain reaction medium tube containing 4.3 mL of medium, and the tube was inverted five times to mix.

Matched cervical liquid-based cytology (ThinPrep®) samples were obtained during the speculum examination before colposcopy. Participants were asked to complete a questionnaire about the acceptability of the tests performed.

Pseudo-anonymised urine and cervical samples were delivered to the MFT virology laboratory on the same day as collection. Urine samples were kept at 4°C and cervical samples were stored at room temperature.

2.4 | Laboratory analyses

All laboratory-based analyses were performed by medical laboratory assistants working in the MFT NHS Cervical

Screening Programme Laboratory within 29 days (mean of 6.44 days) of specimen collection. HPV testing was carried out by the Roche cobas® 8800 (liquid-based cytology as per manufacturer's guidance and urine samples tested 'off label' as to date, there are no Conformité Européenne [CE] manufacturer's claims) using the pre-set cycle thresholds (C_t) for HPV type 16, HPV type 18 and HPV Other (HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68), using β-globin DNA as a control for sample cellularity, valid sample extraction and amplification. Cycle thresholds are an indication of viral load, with lower C_t values indicating a higher sample viral load. Initial analyses included all urine samples collected using the two collection devices, irrespective of sample quality. In post hoc analyses, those with insufficient DNA (defined as samples with C_t values greater than the mean cervical sample β-globin level + 3SD cutoff) were considered inadequate and excluded from secondary analyses. Urine samples showing positivity for any HPV type were considered positive even if different HPV types were found on the cervical sample, as positivity for any type would trigger further investigation within the cervical screening programme.

2.5 | Clinical outcomes

Diagnosis was determined by clinical procedures, e.g. colposcopic findings or histology (if applicable). Histological sample results taken on the same day as the research samples determined the final diagnosis, except in cases where the biopsy was a lower grade to the excisional cervical treatment histology. In this case, we inferred that the smaller diagnostic biopsy missed higher-grade disease and therefore final histology was taken as the excisional treatment result.

2.6 | Statistical analysis

The primary outcome was the diagnostic accuracy of HPV-tested urine for CIN2+ detection. Predetermined secondary outcomes compared test accuracy of the two urine collection devices, concordance with cervical samples, and the acceptability of urine-based cervical screening.

A sample size calculation determined that 120 women with CIN2+ in each randomisation arm would give 89.8% power to find that the lower bound of the 95% CI for sensitivity to CIN2+ was ≥80%, assuming that the true sensitivity was 90%. Based on our pilot study results,[8] 480 colposcopy attendees would yield approximately 240 participants with CIN2+.

Descriptive statistics were summarised, and differences were calculated according to data distribution – normally distributed by mean ± SD and paired *t* test, non-normally distributed by median (interquartile range) and Mann–Whitney *U* test. Absolute and relative clinical test accuracy was calculated with accompanying 95% CI. The McNemar test was used to assess differences in sensitivity between paired FVU and cervical samples, and the chi-square test was used to assess differences between sensitivity of FVU-device-collected urine and standard-pot-collected urine. Concordance between urine and cervical samples for HPV positivity was determined using Cohen's κ statistical test and was categorised as follows: $\kappa \leq 0.20$, poor; $0.21 \leq \kappa \leq 0.40$, fair; $0.41 \leq \kappa \leq 0.60$, moderate; $0.61 \leq \kappa \leq 0.80$, good; $\kappa \geq 0.81$, excellent.

Statistical analyses were performed with Stata version 17 (Stata Corp., College Station, TX, USA) and GraphPad Prism (version 9.3.1; GraphPad, San Diego, CA, USA).

3 | RESULTS

3.1 | Study participants and sample characteristics

Between May 2021 and February 2022, 516 participants were assessed for trial eligibility and 480 were randomised 1:1 to an FVU-collection device or to standard-pot-collected urine. The main reasons for non-participation were feeling overwhelmed by the clinical procedures, feeling anxious and being unable to urinate in a public setting on demand. On testing, there was one confirmed invalid non-urine sample provided, all other urine and cervical samples demonstrated test validity. After exclusions based on missing urine and/or matched cervical samples ($n = 15$), 465 matched urine and cervical samples were included in the final analysis (FVU-collection device $n = 235$, standard pot $n = 230$) (Figure 1).

The demographics of the study population were as follows: median age (32 versus 34 years), ethnicity (79% versus 81% white), highest educational level (21% versus 21% GCE/O-level/GCSE [school examinations taken at age 16 years], 17% versus 16% A-level or equivalent [school examinations taken at age 18 years], 24% versus 27% undergraduate, 17% versus 16% postgraduate), employment status (83% versus 82% employed, 6% versus 6% unemployed), sexual orientation (94% versus 92% heterosexual) and referral screening results (44% versus 44% high grade; 43% versus 43% low grade/borderline; and 11% versus 12% persistent HPV+/cytology-negative) (Table S1) in the FVU-collection device and standard pot groups, respectively.

3.2 | HPV concordance for urine and cervical samples

The mean C_t values (Figure S1) were higher in urine samples when compared with the cervical samples, resulting in positive differences based on paired samples in mean $C_{t_{\text{FVU device}}} - C_{t_{\text{cervical}}}$ of 0.7 for β-globin ($p = <0.0001$, $n = 228$), 3.5 for HPV 16 ($p = <0.0001$, $n = 55$), 3.4 for HPV 18 ($p = 0.04$, $n = 15$), and 3.4 for HPV Other ($p = <0.0001$, $n = 136$); and for mean $C_{t_{\text{standard pot}}} - C_{t_{\text{cervical}}}$ of 3.8 for β-globin ($p = <0.0001$, $n = 228$), 5.4 for HPV 16 ($p = <0.0001$, $n = 47$), 5.4 for HPV 18 ($p = <0.0001$, $n = 16$) and 5.3 for HPV Other ($p = <0.0001$,

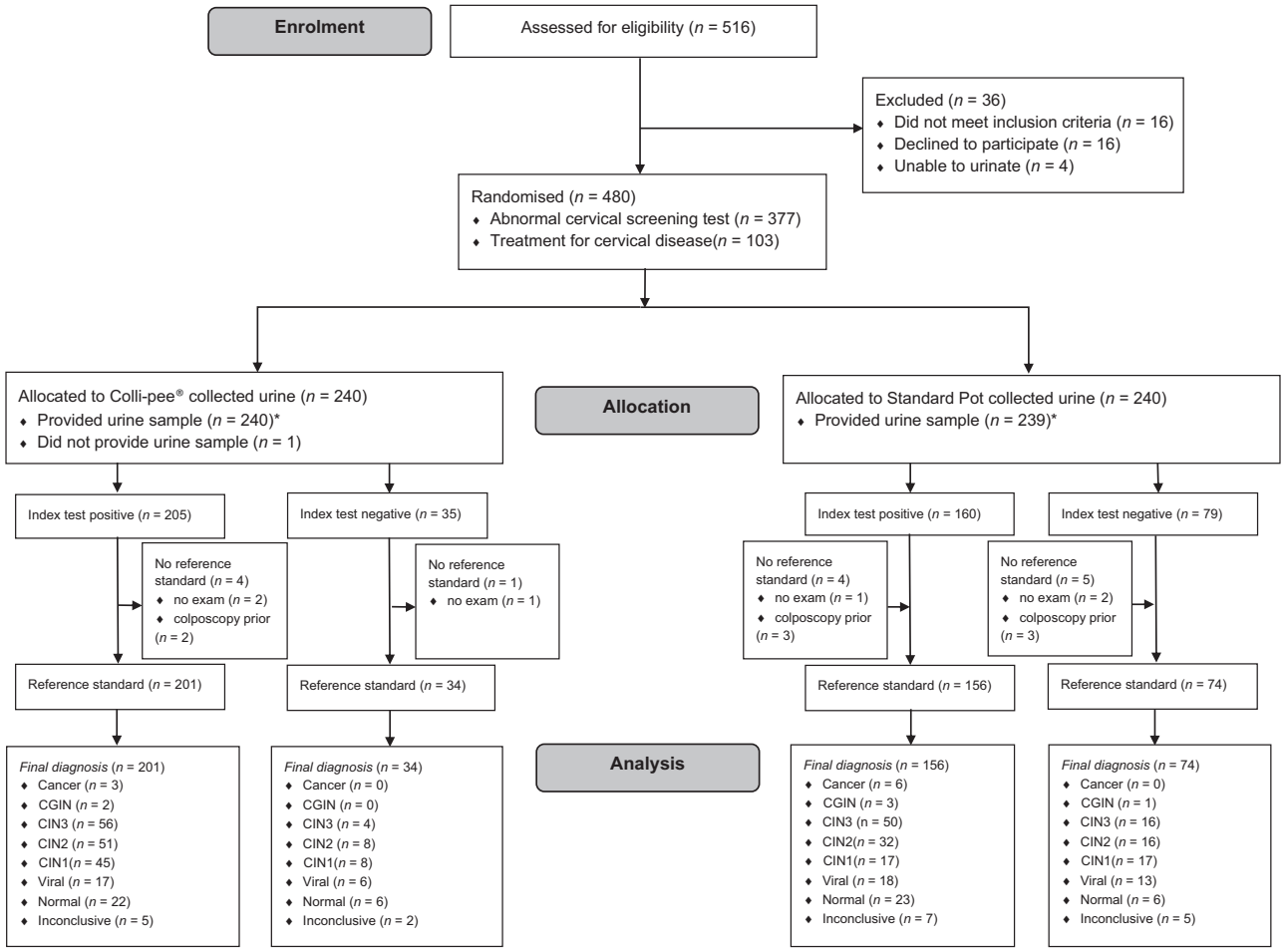

FIGURE 1 STARD diagram showing flow of participants through the study. *One incorrect urine collection device given to participant – FVU collection device given instead of standard pot. Index test, high-risk human papillomavirus (hr-HPV) testing of urine samples; no exam, indicated that no speculum examination was performed and therefore a research cervical sample was not obtained; colposcopy prior, indicates that a cervical sample was unable to be taken as colposcopic solutions had already been applied to the cervix and therefore cervical sample accuracy could be compromised; reference standard, hr-HPV testing of cervical samples. Final diagnosis: CGIN, cervical glandular intraepithelial hyperplasia; CIN, cervical intraepithelial hyperplasia (grades 1–3); viral, HPV infection; inconclusive, no final diagnosis made as colposcopy inadequate and no histology obtained.

$n = 95$). C_t values were significantly higher for standard-pot-collected urine than FVU-device-collected urine for β-globin, HPV 16 and HPV Other; $C_{t_{\text{standard pot}}} - C_{t_{\text{FVU device}}}$ of 3.1 for β-globin ($p = <0.0001$), 1.8 for HPV 16 ($p = 0.04$), 0.006 for HPV 18 ($p = >0.9$) and 1.2 for HPV Other ($p = 0.02$).

Table S2 shows moderate HPV concordance between FVU device-collected urine and matched cervical samples ($\kappa = 0.49$; 95% CI 0.32–0.65) and fair concordance between standard-pot-collected urine and matched cervical sampling ($\kappa = 0.34$; 95% CI 0.21–0.46).

3.3 | Clinical performance of Roche cobas 8800 HPV testing

Final diagnosis was available for 97.0% ($n = 228/235$; 194 by histology and 34 by colposcopic impression) in the FVU-collection device group and 94.8% ($n = 218/230$; 179 by histology and 39 by colposcopic impression) in the standard pot group. Where final diagnosis was classified as normal, viral or CIN1, an overall classification of less than CIN2 was applied. Where final diagnosis was ungraded CIN, CIN2, CIN3, cervical glandular intraepithelial neoplasia (CGIN) and cancer, an overall classification of CIN2+ was applied. Where final diagnosis was CIN3, CGIN and cancer, an overall classification of CIN3+ was applied.

When applying the manufacturer's cycle threshold cut-off for cervical sample testing, the FVU-collection device was both more sensitive for detecting CIN2+ (90.3% versus 73.4%, χ^2 $p = 0.0005$) and CIN3+ (93.8% versus 77.6%, χ^2 $p = 0.007$) than the standard pot, respectively (Table 1). Six of the 228 CIN2+ cases were missed by HPV testing in cervical samples (three CIN2, two CIN3 and one ungraded CIN), 12/124 CIN2+ cases were missed in the FVU-collection device arm (seven CIN2, four CIN3 and one ungraded CIN) and 33/124 CIN2+ cases were missed in the standard pot arm (13 CIN2, 16 CIN3, 1 CGIN, 3 ungraded CIN).

The relative clinical sensitivity of HPV-tested FVU-device-collected urine when compared with matched cervical samples was 0.92 (ratio; 95% CI 0.87–0.97; $p_{\text{McN}} = 0.004$)

TABLE 1 Absolute and relative clinical sensitivity (CIN2+ and CIN3+) and specificity (<CIN2) of the Roche cobas 8800 high-risk HPV assay in cervical samples and first-void urine (FVU) collected with the FVU collection device and standard pot.

	CIN2+			CIN3+			<CIN2		
	n	Sensitivity % (95% CI)	Relative Sensitivity[a] ratio (95% CI) *p* value[b]	*n*	Sensitivity % (95% CI)	Relative sensitivity[a] ratio (95% CI) *p* value[b]	*n*	Specificity % (95% CI)	Relative Specificity[a] ratio (95% CI) *p* value[b]
Cervical samples	242/248	97.58 (94.81–99.11)		139/141	98.58 (94.97–99.83)		40/198	20.20 (14.84–26.47)	
FVU-collection device	112/124	90.32 (83.71–94.90)	0.92 (0.87–0.97) $p = 0.004$	61/65	93.85 (84.99–98.30)	0.95 (0.89–1.02) $p = 0.08$	20/104	19.23 (12.16–28.12)	1.33 (0.92–1.94) $p = 0.13$
Standard pot	91/124	73.39 (64.70–80.91)	0.76 (0.68–0.84) $p < 0.0001$	59/76	77.63 (66.62–86.40)	0.79 (0.70–0.89) $p = 0.0002$	36/94	38.30 (28.46–48.89)	1.44 (1.05–1.97) $p = 0.02$

Abbreviations: 95% CI, exact 95% confidence interval; CIN, cervical intraepithelial neoplasia; FVU, first-void urine; HPV, human papillomavirus.

[a]Relative sensitivity and specificity are restricted to the groups of paired samples: 228 women with paired cervical and FVU-device-collected samples (sensitivity 98.39% and specificity 14.42% for the cervical samples among these 228 women) and 218 women with paired cervical and standard pot samples (sensitivity 96.77% and specificity 26.60% for the cervical samples among these 218 women). Further data are shown in Table S2.

[b]McNemar's paired *p* value.

and standard-pot-collected urine was 0.76 (ratio; 95% CI 0.68–0.84; $p_{McN} = <0.0001$) (Table 1), indicating that HPV testing for both urine collection methods was less sensitive for detecting CIN2+ than cervical sampling. The relative clinical sensitivity improved for CIN3+ cases in the FVU-collection device arm (ratio = 0.95; 95% CI 0.89–1.02, $p_{McN} = 0.08$) and although the specificity was low it was found to be similarly specific to cervical sampling (ratio = 1.33; 95% CI 0.92–1.94, $p_{McN} = 0.13$).

There were 161 participants in the FVU-collection device arm aged 30 years or older. In this subgroup analysis the absolute sensitivity of urine HPV testing for CIN2+ was 90.0% (95% CI 81.9%–95.3%) and cervical was 98.9% (95% CI 94.0%–100.0%). The relative sensitivity of urine HPV testing for CIN2+ was 0.91 (ratio; 95% CI 0.85%–0.97%; $p_{McN} = 0.005$).

Post-hoc analysis scrutinising sample adequacy for testing found a cervical mean β-globin C_t of 25.33 ($n = 459$) and an SD of 1.637. The mean + 3SD for a β-globin cutoff accuracy measure was 30.24. Applying this cutoff to the FVU-device-collected urine found four samples to be inadequate, of which two had CIN2+. Removing the inadequate samples from the analysis showed test sensitivity for CIN2+ detection to be 91.8% (95% CI 85.4%–96.0%) and relative sensitivity to be 0.93 (95% CI 0.88%–0.98%). In standard-pot-collected urine, 72 samples were inadequate, the removal of which gave an adjusted test sensitivity for CIN2+ detection of 80.0% (95% CI 70.3%–87.7%) and relative sensitivity of 0.83 (95% CI 0.75%–0.92%), compared with cervical sampling.

3.4 | Acceptability of cervical screening methods

A total of 465 participants answered the acceptability questionnaire. 76% had not heard of HPV previously despite being referred for colposcopy due to an HPV-positive cervical screen. Participants were confident to obtain a urine sample for cervical screening using both urine collection devices; however, a higher number of participants found the FVU-collection device easier than the standard pot (99.6% versus 94.5%) (Figures 2 and 3). Both arms showed slight differences in preference for future screening methods; preference for urine screening (40.0% versus 31.7%), no preference (37.0% versus 37.4%) and preference for a clinician-collected cervical sample (22.0% versus 30.0%), in the in FVU-collection device and standard pot arms, respectively.

4 | DISCUSSION

4.1 | Main findings

We studied urine HPV testing for CIN2+ detection in a colposcopy referral population, comparing two urine collection devices, using the Roche cobas® 8800 HPV assay. We showed

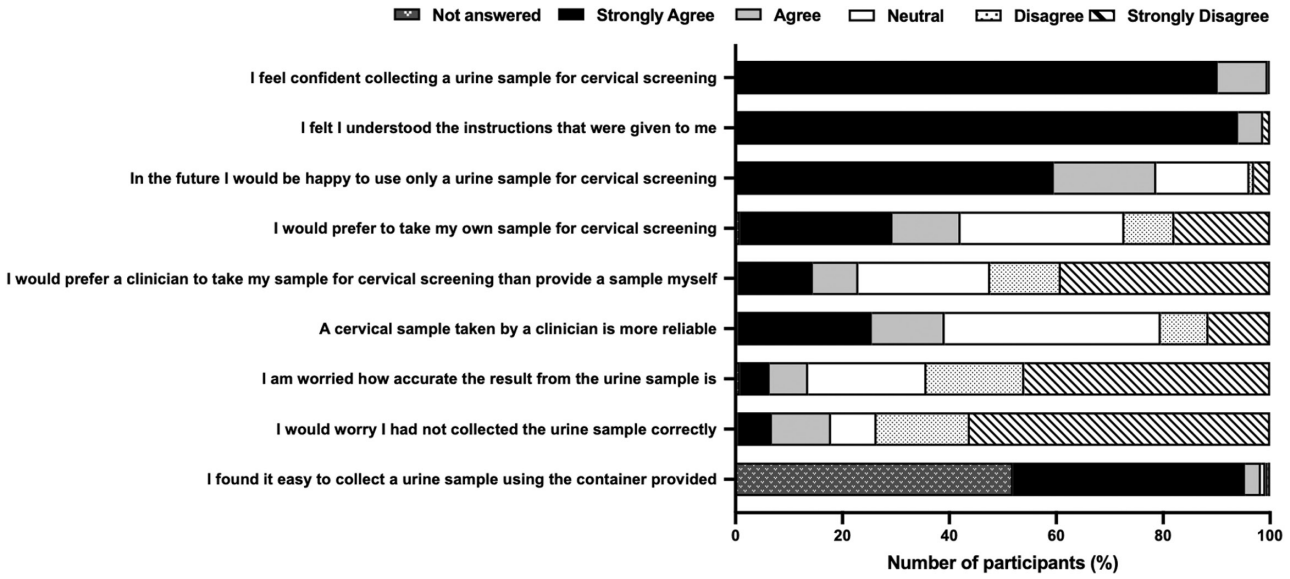

FIGURE 2 The acceptability of first-void urine (FVU) -device-collected urine for cervical screening.

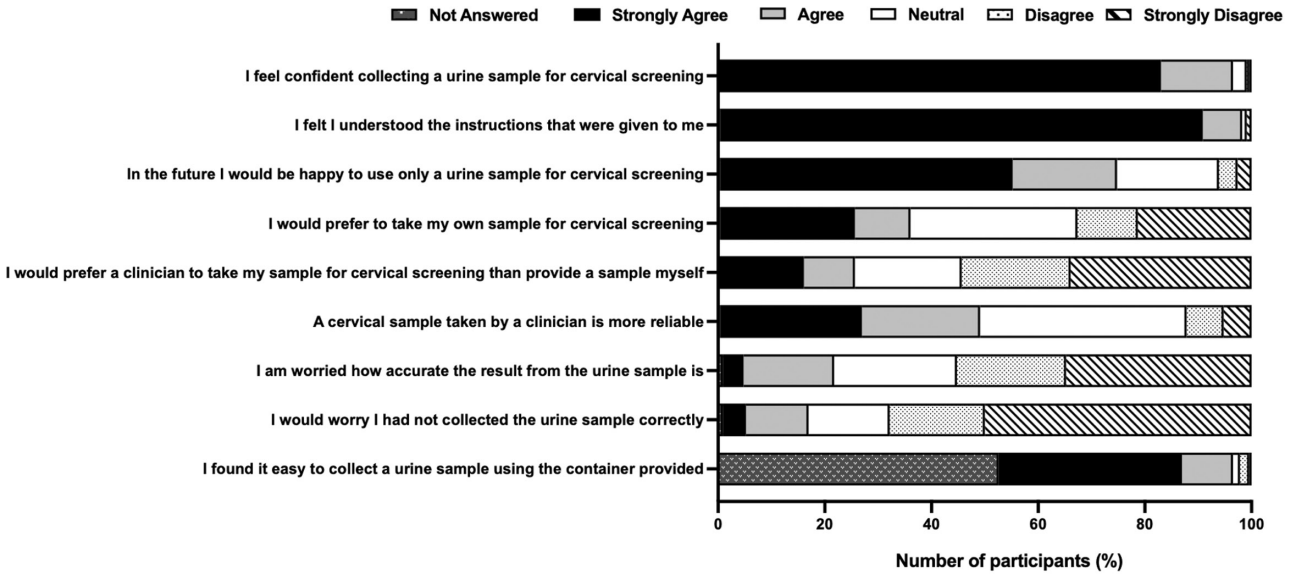

FIGURE 3 The acceptability of standard-pot-collected urine for cervical screening.

that HPV-tested urine collected using the FVU-collection device has superior sensitivity for CIN2+ detection than standard-pot-collected urine (90.3% versus 73.4%). Urine collected with an FVU-collection device has promising sensitivity for CIN2+ detection, with a relative sensitivity of 0.92 (95% CI 0.87–0.97) compared with clinician-obtained cervical samples. The standard pot performed worse, with a relative sensitivity of 0.76 (95% CI 0.68–0.84). Removing urine samples that did not reach the β-globin sample adequacy cutoff improved sensitivity further to 91.8% (95% CI 85.4%–96.0%, relative sensitivity 0.93) and 80.0% (95% CI 70.3%–87.7%, relative sensitivity 0.83) in the FVU-collection device and standard pot arms, respectively. These data suggest that urine sample adequacy is an important consideration for optimising test accuracy. Urine-based self-sampling was broadly acceptable to this colposcopy referral population. Future studies should test the clinical performance of urine collected with an FVU-collection device in a general screening population and explore its potential to improve cervical screening uptake in current non-attenders.

4.2 | Strengths and limitations

To our knowledge this is the only randomised controlled trial evaluating HPV testing of urine collected by two different devices, enabling a rigorous direct comparison. This study justifies the use of a specialised FVU-collection device for HPV testing, despite the possible increased costs and environmental impact associated with its use compared with

the standard pot. The reduction in pot-collected urine sensitivity could be in part the result of the higher urine volume collected using this method (approximately 40 mL versus 6.6 mL in the FVU-collection device and standard pot, respectively). The FVU-collection device reliably collects the first 6.6 mL of the urine because of the overflow valve, which enables the rest of the void to be discarded, improving the ability of the user to collect the first fraction of urine flow compared with the standard pot. Our study has the largest number of CIN2+ cases of all urine HPV testing studies in the literature, facilitating a thorough assessment of its clinical sensitivity. Matched, same-day urine and cervical samples were compared, minimising the risk of discordance due to changes in HPV infection status between samples. HPV testing used the NHS Cervical Screening Programme-approved Roche cobas® 8800, enabling direct translatability to the current cervical screening programme.

Limitations of the study relate to its colposcopy clinic setting. We do not know how urine HPV testing will perform in the general screening population where interventions to improve cervical screening uptake are most needed. As urine was stored in the fridge, we cannot attest to the stability of urine collected in community settings and left at ambient temperature for HPV detection. However, urine stability in preservative is likely to be similar to that of cervical and vaginal samples with the Becton Dickinson (BD) Instructions for Use stating that samples are stable for 30 days at 30°C or for 6 days at 40°C.[17] Only one HPV assay was used in this study and there are multiple others in routine NHS use requiring validation. HPV-tested FVU-device-collected urine had sensitivity relative to that of cervical samples for CIN2+ detection, outperforming cytology (relative sensitivity of liquid-based cytology versus HC2 HPV assay = 0.82),[18] which was the primary screening test in the NHS Cervical Screening Programme until 2019, although no direct comparison was performed in this study. Use of bespoke urine HPV and/or β-globin C_t thresholds is likely to further improve test sensitivity. Specificity was low across all sample types because of the high burden of HPV in this population, all referred as they had had a positive HPV cervical screening test originally. This population has an inherent bias positively inflating HPV test accuracy of a cervical sample and urine will always fall short within this setting. Although the FVU-collection device arm showed comparable specificity (19.2%) to cervical sampling (20.2%) for CIN2+ detection, further evaluation in a general screening population is needed to ascertain true test performance.

4.3 | Interpretation

This study, alongside the VALHUDES, PREDICTORS 5.1 and EVAH studies,[6,12,19,20] show that urine HPV testing has real world potential for clinical implementation. When a specialised FVU-collection device is used, a reliable and standardised small volume of FVU is collected and stabilised with a preservative, optimising test accuracy, making it a reasonable alternative to cervical sampling for CIN2+ detection. In our colposcopy population, after applying a urine β-globin C_t threshold for sample adequacy, we obtained a CIN2+ detection relative sensitivity of 0.93 (95% CI 0.88–0.98) for FVU-device-collected urine compared with cervical sampling, similar to the 0.95 (95% CI 0.88–1.01) reported in the VALHUDES study that HPV tested using Abbott RealTime®.[6] VALHUDES showed a higher relative sensitivity of 1.00 (95% CI 0.95–1.05) at an exploratory higher C_t positivity cutoff for urine. Here, instead of bespoke HPV positivity C_t cutoffs to improve test accuracy, we highlight an alternative strategy of excluding inadequately cellular samples to improve test reliability. If an inadequacy cutoff were used we would expect 3% of urine samples to be 'rejected' with a repeat urine or cervical sample advised in this setting. The VALHUDES samples were also HPV tested by BD Onclarity®, which gave a higher relative sensitivity of 1.00 (95% CI 0.93–1.07).[12] It is, however, important to note that in the VALHUDES studies the HPV-tested cervical sample clinical sensitivity for CIN2+ was 93.2% and that of urine sampling was 88.6% ($n = 88$) using Abbott RealTime® and cervical and urine clinical sensitivity was 90.9% using BD Onclarity®; both of which are lower than in this study using the Roche cobas® 8800. Other HPV polymerase chain reaction assays need to be validated for urine-based testing, including those using mRNA-based technology, as a range of different tests are approved for use in the NHS and other national cervical screening programmes.

We found that optimising urine collection with the FVU-collection device significantly improved test sensitivity compared with a standard pot ($p = 0.0005$ for CIN2+). This is the first study to use a 10-mL FVU-collection device and it is reassuring that it shows comparable test sensitivity to the 20-mL collection device.[6,19,20] This also highlights that test accuracy is not compromised by obtaining 13 mL of FVU. The 10-mL device is compact, contains just 3.4 mL non-toxic UCM preservative for urine stabilisation, and can be posted through a standard UK letterbox to facilitate at-home testing.

Urine self-sampling was acceptable to women attending the colposcopy clinic, which is consistent with other studies.[21–23] Very few women declined participation, but the most common reason was anxiety relating to their colposcopy. The most common reason for withdrawal was inability to urinate for the purposes of the study (Figure 1). Neither scenario would prevent home-based urine sampling for cervical screening. The high ease of use reported in the FVU-collection device arm is encouraging, indicating that the device is likely to be acceptable within the general screening population too. Self-sampling is also available with a vaginal swab, which has been adopted as the primary screening method in at least 9 countries around the world[24] because of its comparable test accuracy to routine screening,[25] likely ability to increase screening numbers[26] and cost-effectiveness.[15] However, UK-based studies assessing uptake among non-attenders showed at best a 10% uplift with the offer of vaginal self-sampling,[27,28] implying that not all attendance barriers are met.[29] Adequate vaginal self-sample acquisition remains a user concern that needs to be further addressed.[30]

The true value of urine-based cervical screening is likely to be its ability to improve cervical screening uptake in current non-attenders[31] through eliminating the barriers of inconvenience, access, embarrassment and fear.[32] Urine-based screening is likely to reduce the financial and environmental costs associated with cervical sampling, including appointments with healthcare professionals and use of specialised equipment.[14,15] It also offers non-contact screening during future pandemics such as that experienced with SARS-CoV-2. In countries where there is no organised screening programme and the burden of disease is greatest, urine sampling offers the potential for an acceptable, cost-effective, point-of-care self-sampling test, enabling targeted further investigation and treatment on the same day to minimise loss to follow up.

The disadvantage of self-sampling methods is that reflex triage cytology is not possible,[33] which currently necessitates a routine cervical sample for clinical management decisions. Encouragingly, those who screen positive for HPV through self-sampling are very likely to attend for cervical sampling when called.[34] Molecular triage methods, for example extended genotyping, HPV integration testing and methylation analysis, show promising results to further triage self-sampled HPV-positive individuals for further investigation[35–37] and work in this area continues apace.

5 | CONCLUSION

HPV testing of urine collected with an FVU collection device has promising sensitivity for CIN2+ detection in a colposcopy population. Defining urine sample adequacy through β-globin C_t thresholds improves test reliability. Standard-pot-collected urine has insufficient sensitivity to detect CIN2+ and cannot be recommended for routine cervical screening. Urine-based cervical screening is more acceptable than cervical sampling in colposcopy clinic attendees. Testing within the general screening population is now imperative to further delineate its feasibility, usability and clinical performance, including long-term outcomes of any urine-positive/cervical-negative cases. Non-attenders of cervical screening require additional focus because urine sampling could overcome many of their barriers to screening. Urine-based screening could provide an urgent solution to resource-poor settings lacking organised and effective screening programmes, accelerating WHO's target of the elimination of cervical cancer in the next decade.[38]

AUTHOR CONTRIBUTIONS

EJC was Principal Investigator, obtained funding and is the study guarantor. EJC conceived and designed the study with support from PS. JCD screened and recruited all participants. AS supervised the high-risk HPV testing of all samples. JCD, SC and EP acquired the data and checked for accuracy. JCD performed statistical analysis under the guidance of CG. JCD wrote the first draft of the manuscript. All authors reviewed the manuscript and approved the final version for publication.

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CONFLICT OF INTEREST STATEMENT

Roche provided this study with the testing kits to undergo testing with the Roche Cobas 8800 hr-HPV assay. Novosanis provided the Colli-pee urine collection devices for the study. There are no other conflicts of interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The study was approved by the North-West Greater Manchester Research Ethics Committee (20/NW/0389) on 16 November 2020 and registered as a clinical trial (ISRCTN13132810). The study was conducted in accordance with the GCP, GDPR and Declaration of Helsinki.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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