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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-025388
Article Type:	Research
Date Submitted by the Author:	12-Jul-2018
Complete List of Authors:	Sargent, Alex; Manchester University NHS Foundation Trust, Clinical Virology Department Fletcher, Samantha; University of Manchester Faculty of Biology, Medicine and Health, Gynaecological Oncology Bray, Katarina; University of Manchester, Oxford Road, M13 9PL, Manchester, UK, Division of Cancer Sciences Kitchener, Henry; University of Manchester, Oxford Road, M13 9PL, Manchester, UK, Division of Cancer Sciences Crosbie, Emma; University of Manchester Faculty of Biology, Medicine and Health, Gynaecological Oncology
Keywords:	Urinary human papillomavirus (HPV) detection, Cervical intraepithelial neoplasia (CIN), Cervical cancer screening, Acceptability

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An exploratory study of HPV testing in self-sampled urine and comparison with matched vaginal and cervical samples

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Word count: abstract 309 words, manuscript 2664 words, 2 tables, 3 figures, supplemental material

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ABSTRACT

Objectives: Human papillomavirus (HPV) testing in cervical screening offers the potential for self-sampling to improve uptake amongst non-attenders. High-risk (HR) HPV detection in urine shows promise, but few studies have examined its sensitivity for CIN2+ detection compared with standard cervical samples. The aims of this exploratory study were to optimize conditions for urine testing for HPV detection; to determine concordance for HR-HPV detection in matched urine, vaginal and cervical samples; to compare the sensitivity of HR-HPV testing for the detection of CIN2+ in matched samples; and to determine the acceptability of urine testing for cervical screening.

Design: Prospective cohort study

Setting: Secondary care colposcopy clinic in North West England

Participants: Women aged 25 years of age or older, attending colposcopy clinic for management of abnormal cervical screening results or a suspicious-looking cervix. In total, 104 women took part in the study. Triple matched samples were available for 79 and 66 women using Abbott RealTi*m*e (ART) and Roche Cobas 4800 (RC) respectively.

Intervention: Self-collected urine and vaginal samples and practitioner-obtained cervical samples were tested for HR-HPV by ART and RC assays, including comparison of neat and preservative-fixed urine. Colposcopic opinion was recorded and directed cervical biopsies taken if clinically indicated. The acceptability of self-testing was evaluated by questionnaire.

Primary outcome measure: The sensitivity of urine to detect underlying CIN2+

Secondary Outcome Measures: The comparative sensitivity of vaginal and cervical samples to detect CIN2+; the acceptability of urine sampling.

Results: Preservative-fixed, but not neat urine, showed good concordance with vaginal samples for the detection of HR-HPV. The sensitivity for detecting CIN2+ was 15/18 (83%) for urine and 16/18 (89%) for cervical and vaginal samples by ART, and 15/17 (88%) for all samples by RC. Urine-based testing was broadly acceptable to women.

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Conclusions: Urinary HR-HPV detection offers an alternative strategy of cervical screening. Larger studies to determine its clinical utility are warranted.

Keywords: Urinary human papillomavirus (HPV) detection, cervical intraepithelial neoplasia (CIN), cervical cancer screening, acceptability

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Strengths and limitations of this study

- Urine HPV testing may circumvent several barriers to cervical screening and this study is therefore timely and important
- We optimised urine HPV testing by comparing neat and preservative-fixed urine using two different HR-HPV detection assays alongside matched vaginal and cervical samples
- We determined the acceptability of urine HPV testing by studying women's preferences for urine self-sampling compared with standard cervical screening and vaginal self-sampling
- A colposcopy clinic population is important when testing the potential of urine HPV detection but a lower prevalence of HR-HPV in the general cervical screening population may affect its clinical performance
- The true value of urine HPV testing must be established in the general screening population and ultimately, in cervical screening non-attenders

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BACKGROUND

The strategy of cervical screening depends on reducing the incidence of cervical cancer through the detection and treatment of its precursor lesion, high grade cervical intraepithelial neoplasia (CIN2+) (1). Cervical cancer is caused by persistent infection with high-risk human papillomavirus (HR-HPV) (2) and a pooled analysis of four large RCTS found that detection of HR-HPV in practitioner-obtained cervical samples is a more sensitive primary screen than cervical cytology for the detection of CIN2+ (3). The UK National Screening Committee (UK NSC), together with other publically funded programs has therefore recommended moving to primary HPV testing for general population screening, with reflex cytology on positive samples.

Despite the success of cervical screening, recent years have witnessed a gradual decline in its uptake, particularly by women aged 25-49 years. Barriers to cervical screening include embarrassment, fear, inconvenience and some women believe that they are not at risk (4). It may be possible to overcome some of these barriers by improving the acceptability of the screening test and the ease with which it can be performed. Vaginal self-sampling kits for HR-HPV detection have a modest impact on screening uptake by chronic non-attenders (5). The diagnostic accuracy of HPV detection in self-obtained vaginal samples, while less than for practitioner-obtained cervical samples, is sufficient to recommend its use in non-attenders and in low resource settings (6).

HPV detection in urine has recently been considered as an alternative means to identify women at increased risk of high grade CIN. A systematic review with meta-analysis found a pooled sensitivity of 77% and specificity of 88% for HR-HPV detection in urine (7). This pooled sensitivity was lower than for HR-HPV detection in practitioner-obtained cervical samples but the wide variation in methodology, including urine sampling techniques, HPV detection methodology and study setting precluded unbiased comparison. Since HPV testing is more sensitive than cytology for the detection

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of CIN2+, a degree of decreased sensitivity of urinary HPV detection could be offset by improved uptake of cervical screening (8).

The aims of this exploratory study were fourfold: to optimize urine processing for HR-HPV detection; to establish the concordance of HR-HPV detection in matched urine, vaginal and cervical samples in a colposcopy population; to compare the sensitivity of HR-HPV testing for CIN2+ detection in the same samples; and to determine the acceptability by women of self-collected versus practitioner-obtained samples for cervical screening.

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METHODS

Study participants and sample collection

Urine, vaginal and cervical samples were collected from women attending the colposcopy clinic at St Mary's Hospital, Manchester University Foundation Trust (MFT) between June and September 2015. A participant information sheet was sent with the colposcopy appointment letter to women who were 25 years of age or older and attending the colposcopy clinic for management of abnormal cervical screening results or a suspicious-looking cervix. The study was approved by the North West Research Ethics Committee (15/NW/0218) and all women gave written, informed consent to take part. Women who declined participation in the study were asked to give their reasons. Pregnant women were excluded from the study. Study samples were obtained prior to colposcopic examination of the cervix, where this was required. Self-collected first stream urine samples were collected in a sterile dry pot, followed by self-collected vaginal samples obtained using the Rovers Evalyn Brush (Netherlands). Women were asked to place the brush in the vagina, rotate it five times for sample collection and replace the brush in its casing. Practitioner-obtained cervical samples were then taken with a cervical broom immediately prior to clinically-indicated procedures. Following colposcopy, women were asked to complete a questionnaire about the acceptability of the tests performed (**Supplemental Appendix 1**).

Clinical procedures

Women were managed clinically according to NHS Cervical Screening Programme guidelines. All colposcopists were accredited for independent practice by the British Society of Colposcopy and Cervical Pathology (BSCCP). Abnormal colposcopy indicated directed punch biopsy and histological review by consultant gynecological histopathologists, who classified biopsies as normal, low grade CIN (CIN1), or high grade CIN (CIN2/3).

HPV DNA testing

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All samples were delivered to the MFT Virology laboratory within four hours of collection. Cervical samples were collected using SurePath Liquid Based Cytology (LBC) and vaginal cells were suspended in solution by agitating the Evalyn brush in SurePath preservative on receipt in the laboratory. Sample aliquots were added to Abbott and Roche secondary tubes as per the manufacturers' instructions. Urine samples were refrigerated at 4 degrees both without preservative ('neat') and following transfer to Abbott and Roche preservative tubes ('preservative-fixed') using the Abbott multi-collect specimen collection kit and the Cobas PCR urine specimen collection kit. Batches of samples were tested for high risk HPV using the Abbott RealTi*m*e (ART) and Roche Cobas 4800 (RC) assays within 48 hours and one week of sample processing, respectively. Both assays used fully automated systems that amplify and detect 14 HR-HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) simultaneously. Separate probes enable the specific detection of HPV types 16 and 18. Both assays also measure human beta globin as a control for sample cellularity, valid sample extraction and amplification. The cycle threshold (ct) is a measure of DNA concentration, with ct values inversely proportional to the amount of target nucleic acid in the sample. All samples were tested according to the manufacturers' instructions.

Statistical analysis

Concordance between the different samples for HR-HPV positivity was determined using Cohen's kappa statistic. Agreement was slight (k<0.20), weak (k=0.21-0.40), moderate (k=0.41-0.60), strong (k=0.61-0.80), near perfect (k=0.81-0.99), or perfect (k=1.00). The sensitivity of HR-HPV positivity for CIN2+ detection was compared between urine, vaginal and cervical samples.

Patient and public involvement

Patients and the public were not involved in designing or conducting this research. We will work with patient support groups and forums, including Jo's Cervical Cancer Trust, to help disseminate the results of this study.

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RESULTS

Study participants

One hundred and four women took part in the study (**Figure 1**). Their median age range and distribution reflected that of routine colposcopic practice, with the large majority under the age of 40 years. Non-participants had similar demographics to those who did agree to the study. The reasons for non-participation are shown in **Figure 1**.

Optimization of urine sample processing for HR-HPV testing

A comparison was made between neat and preservative-fixed urine for HR-HPV detection using both ART and RC assays for the first 50 study participants. There were 44 matched neat and preservative-fixed urine sample pairs available for the ART assay. Two failed the internal beta globin control and were excluded from further analysis. A cycle threshold (ct) cut off of ≤32 was used to define HR-HPV positivity. **Table 1** demonstrates good concordance between neat and preservative-fixed urine samples (37/42, 88%, k=0.76).

TABLE 1: Comparison of neat versus preservative-fixed urine using the Abbott RealTi*m*e (ART) assay and the Roche Cobas 4800 (RC) assay for HR-HPV detection

ART assay	Neat positive	Neat negative	Total
Preservative positive	19	3	22
Preservative negative	2	18	20
Total	21	21	42
RC assay	**Neat positive**	**Neat negative**	**Total**
Preservative positive	14	2	16
Preservative negative	0	6	6
Total	14	8	22

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Repeat testing of the 47 preservative-fixed urine samples approximately one month later (mean 33 days) showed no reduction in stability of HR-HPV DNA, with almost identical ct values, as shown in **Supplemental Figure 1** (mean ct 25.2 for samples tested within 48 hours versus 25.1 for samples tested at one month). This was true when all HR-HPV types, HPV 16/18 and HR-HPV other were considered separately (data not shown).

There were 40 matched neat and preservative-fixed urine sample pairs available for the RC assay. Eighteen of the neat but none of the preservative-fixed urine samples, which had been stored for up to one week prior to testing, failed the internal beta globin control and were excluded from further analysis. Thus there were 22 matched urine sample pairs available for testing with the RC assay. A cycle threshold of ≤40 was used to define HR-HPV positivity. **Table 1** shows good concordance between neat and preservative-fixed urine samples (20/22, 90%, k=0.79). Based on these data and the apparent instability of neat urine samples over time, all subsequent urine samples were fixed with preservative upon receipt in the lab, and neat urine samples were not considered further.

HR-HPV detection in urine, vaginal and cervical samples

Abbott RealTi*m*e assay

Seventy-nine participants had valid HR-HPV results from matched urine, vaginal and cervical samples for the ART assay. Directed cervical biopsies were taken from 66 (84%) of these women because of abnormal cytology. Histological analysis classified the biopsies within normal limits (31/66, 47%), CIN1 (17/66, 26%), CIN2 (10/66, 15%) and CIN3 (8/66, 12%).

HR-HPV was detected in 48/79 (61%), 54/79 (68%) and 56/79 (71%) of urine, vaginal and cervical samples respectively. HPV 16/18 was detected in 21/79 (27%), 24/79 (30%) and 23/79 (29%). Concordance between the three sample types was found in 62/79 (79%), as shown in **Table 2**. According to the manufacturer's cut-off of 32ct, the sensitivity for detecting CIN2+ was 16/18 (89%)

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for both cervical and vaginal samples and 15/18 (83%) for urine samples. Concordance between urine and cervical samples was 82% (65/79; k=0.61; 95% CI 0.43-0.79) and between vaginal and cervical samples was 90% (71/79; k=0.76; 95% CI 0.61-0.92).

TABLE 2: HR-HPV detection in preservative-fixed urine, vaginal and cervical samples by the Abbott RealTi*me* (ART) assay and the Roche Cobas 4800 (RC) assay

		Cervical positive		Cervical negative	
		Vaginal positive	**Vaginal negative**	**Vaginal positive**	**Vaginal negative**
ART	**Urine positive**	43[a]	2	2	1
	Urine negative	8[b]	3	1	19[c]
RC					
	Urine positive	44[d]	0	1	0
	Urine negative	4	0	1	16[e]

[a]9 CIN2, 6 CIN3; [b]1 CIN3; [c]1 CIN2, 1 CIN3; [d]8 CIN2, 7 CIN3; [e]1 CIN2, 1 CIN3

A comparison of the mean ct values for detection of the internal beta globin control demonstrated lower DNA concentrations in urine compared with vaginal and cervical samples (**Figure 2a**). The mean ct value for urine, vaginal and cervical samples was 25.6, 20.9 and 23.1 respectively, with similar results for samples which showed positive results for HPV 16/18 or HPV other (25.3, 20.6 and 20.9 respectively) (**Figure 2b**).

Roche Cobas 4800 assay

Sixty-six participants had valid HR-HPV results from matched urine, vaginal and cervical samples for the RC assay. Cervical biopsies were taken for 54 (82%) of these women and histological analysis classified these as normal (24/54, 44%), CIN1 (14/54, 26%), CIN2 (8/54, 15%) and CIN3 (8/54, 15%).

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HR-HPV was detected in 45/66 (68%), 50/66 (76%) and 48/66 (73%) of urine, vaginal and cervical samples respectively. HPV 16/18 was detected in 16/66 (24%), 21/66 (32%) and 19/66 (29%). Concordance between the three sample types was found in 60/66 (91%), as shown in **Table 2**. According to the manufacturer's cut-off of 40ct, the sensitivity for detecting CIN2+ was 15/17 (88%) for all three sample types. Concordance between urine and cervical samples was 92% (61/66, k=0.82, 95% CI 0.67–0.97) and between vaginal and cervical samples was 97% (64/66, k=0.77, 95% CI 0.60-0.92).

A comparison of the mean ct values for detection of the internal beta globin control again demonstrated lower DNA concentrations in urine (**Figure 2c**). The mean ct values for urine, vaginal and cervical samples were 31.7, 27.5 and 29.9 respectively, with similar findings with concordant positive results for HPV 16/18 or HPV other (33.6, 28.1 and 27.7 respectively) (**Figure 2d**).

Acceptability of urine, vaginal and cervical sampling for cervical screening

A total of 98 women answered the acceptability questionnaire (**Supplemental Appendix 1**). Most of the participants had heard about HPV before (yes, 73; no, 17; don't know, 1; not answered, 7). Participants were confident about providing self-obtained vaginal and urine samples for HPV testing and were not excessively concerned about the accuracy of the test in these samples (**Figure 3**). In general, women were more confident about providing a urine sample than a vaginal sample for HPV testing. When asked which sample they would prefer to provide, more women chose urine (39) than either vaginal (32) or cervical samples (17; no preference, 1; not answered, 9).

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DISCUSSION

This study found good concordance for HR-HPV detection between preservative-fixed urine, vaginal and cervical samples amongst women attending a colposcopy clinic. Urine showed similar sensitivity to matched vaginal and cervical samples for CIN2+ detection using both ART and RC assays. Cervical screening by urinary HR-HPV detection was considered acceptable by women.

This was an exploratory study of women attending the colposcopy clinic for the management of abnormal cervical screening results or a suspicious-looking cervix. The prevalence of HR-HPV was therefore sufficiently high to allow comparison between the three sampling methods. Matched urine, vaginal and cervical samples taken at the same visit and immediately prior to colposcopic assessment, eliminated the possibility that HPV had been acquired or cleared between tests. The order of obtaining matched samples meant that cross-contamination was unlikely. We found that preservative-fixed urine was suitable for HR-HPV detection by both ART and RC assays within 48 hours and up to one month following its receipt. The consistent results showed that preservative-fixed urine can be handled in the same way as cervical samples without compromising analytical sensitivity.

The relatively small size of the study means that urine testing needs to be fully validated in larger cohorts. The colposcopy clinic setting facilitated an assessment of the analytical sensitivity of urinary HR-HPV testing but the true value of urine-based cervical screening is likely to be its ability to improve cervical screening uptake by non-attenders. The prevalence of HR-HPV is much lower in the general population, which could be expected to affect the sensitivity and negative predictive value of the test. Studies designed to evaluate the accuracy and clinical utility of urinary HR-HPV detection in the primary cervical screening population are therefore required (9). Urinary self-sampling was acceptable to women attending colposcopy clinic; those who declined participation did so for other reasons. What remains unclear is the acceptability of the test to cervical screening non-attenders

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and the ability of urinary HR-HPV detection to improve uptake. Defining its acceptability in 'hard to reach' groups is challenging but crucial to its further development as an alternative test for cervical screening.

Previous studies have looked at urine sampling techniques (10, 11) but few have reported the optimization of urine processing and storage for HR-HPV detection. Here we show that preservative-fixed urine has improved stability and can be stored prior to testing for much longer than neat urine, which rapidly deteriorates after 48 hours. This may explain the improved clinical performance of urinary HR-HPV reported here compared to studies that tested neat urine (12). In total, 45% of neat urine samples tested on the RC assay at one week were invalid compared to only 2% of samples tested within 48 hours of collection by ART assay. This can be explained by an increase in PCR inhibitors over time. Preservative-fixed urine stored at 4^{o}C showed only one invalid result using the ART assay amongst 104 urine samples tested within 48 hours, and there were no invalid results using the RC assay amongst 98 urine samples tested between 7 and 14 days. Repeat testing at one month showed consistent results.

The analytical and clinical sensitivity of the RC and ART assays for cervical HR-HPV detection are well documented (13-15). The data in this pilot study are consistent with published studies. Raising the threshold above 32ct (manufacturer's cut-off) did pick up four more positive urine samples but did not identify the single undetected CIN2+, which suggests that the manufacturer's cut-offs are appropriate for urine samples. The analytical sensitivity for HR-HPV in urine, vaginal and cervical samples were similar though a lower proportion of HR-HPV was detected in urine samples compared with vaginal samples.

Barriers to cervical screening include embarrassment, fear, inconvenience and the view held by some women that they are not at risk (4). Offering an alternative means of cervical screening can

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encourage non-attenders to be screened, either by 'nudging' them to undergo cervical sampling or by facilitating self-sampling at home (5). Urine-based testing is already widely used for the detection of chlamydia and is generally trusted and acceptable to women. The disadvantage of self-testing is that reflex cytology is not possible in the event of a positive HR-HPV test, however, data suggest that women who screen positive for HR-HPV are highly likely to attend for cervical cytology (16). Urine-based testing may also be cheaper than standard cervical screening tests because it does not require an appointment, a practitioner or any special equipment and it lends itself to high throughput testing. Thus it could also be suitable in resource poor settings where the infrastructure required to underpin screening by cervical cytology is lacking.

CONCLUSIONS

Urinary HR-HPV testing has good sensitivity for the detection of CIN2+ in a colposcopy clinic cohort. Future studies are warranted to assess its performance in the general screening population and its acceptability to women, particularly amongst those who currently do not attend for screening.

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LIST OF ABBREVIATIONS

ART	Abbott RealTi*me*
BSCCP	British Society of Colposcopy and Cervical Pathology
CIN	cervical intraepithelial neoplasia
ct	cycle threshold
HR	high risk
HPV	human papillomavirus
LBC	liquid based cytology
PCR	polymerase chain reaction
RC	Roche Cobas 4800
UK NSC	UK National Screening Committee

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DECLARATIONS

Ethics approval and consent to participate

The study was approved by the North West Research Ethics Committee (15/NW/0218) and all women gave written, informed consent to take part.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

HK is Chair of the Advisory Committee for Cervical Screening (PHE), but the views expressed in this manuscript are those of the author and not Public Health England.

Funding

EC is a National Institute for Health Research (NIHR) Clinician Scientist (NIHR-CS-012-009). This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Author contributions

AS performed the laboratory analyses, data interpretation and wrote the first draft of the manuscript. SF and KB recruited and sampled the study participants and contributed to data interpretation. HK and EC designed the study and contributed to data interpretation and manuscript preparation. All authors reviewed the final version of the manuscript.

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Acknowledgements

The authors would like to thank all the women who participated in this study and donated their samples and data for research. We would like to thank Mrs. Linsey Nelson for her support with obtaining all the necessary regulatory approvals and for data management.

Data sharing statement

Data are available and will be shared upon reasonable request.

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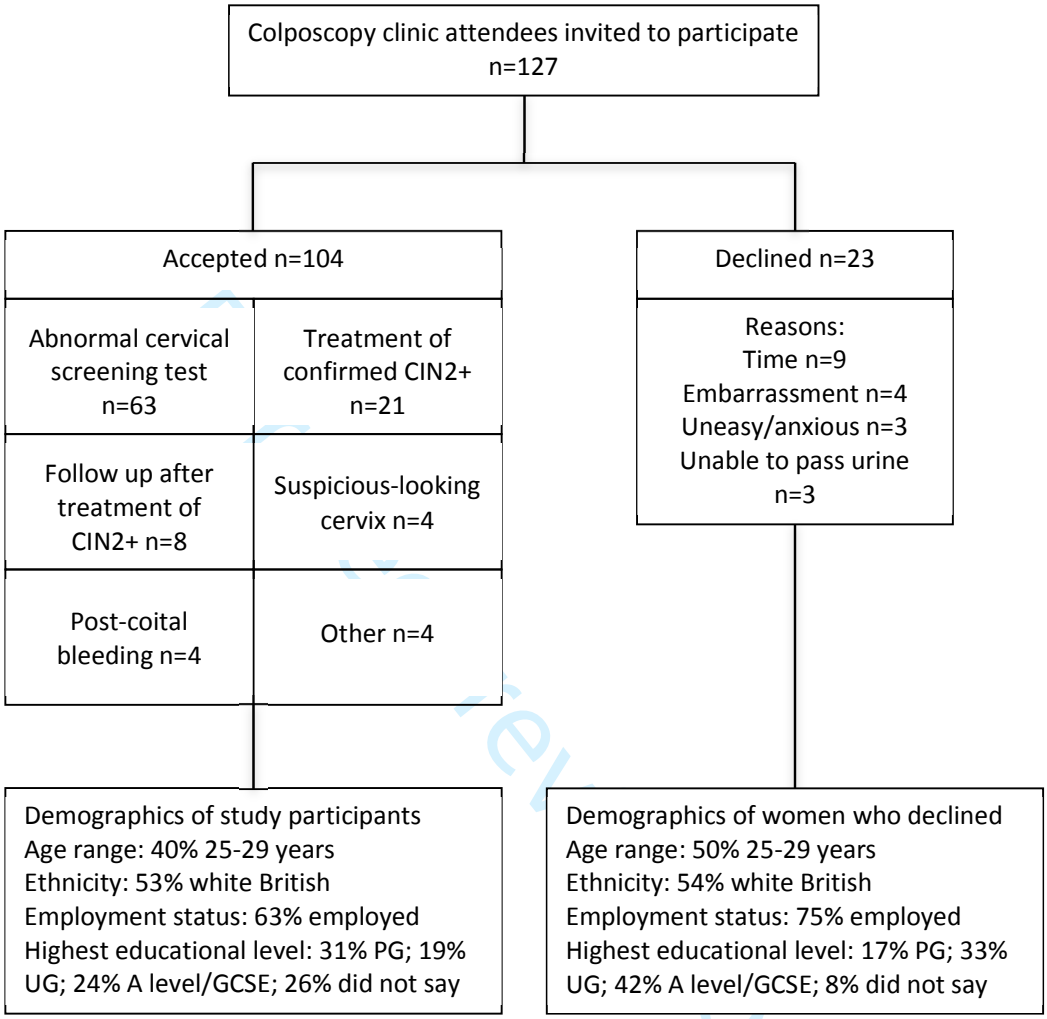
FIGURE 1: Demographics of study participants and reasons for declining participation



PG, postgraduate; UG, undergraduate

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FIGURE 2: A comparison of the mean ct values for detection of the internal beta globin control and HR-HPV in urine, vaginal and cervical samples using the ART (a, b) and RC assays (c, d).

Figure 2a

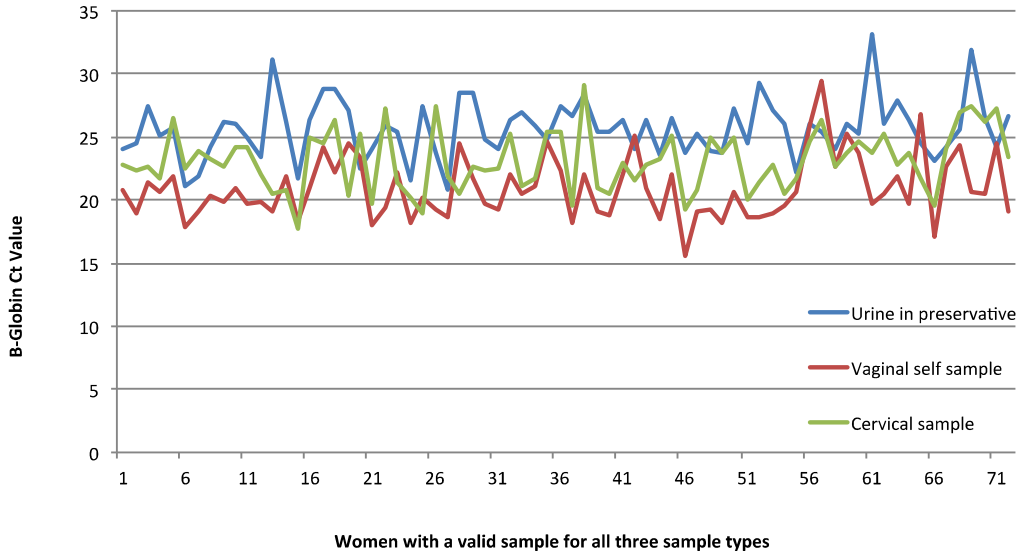

Figure 2b

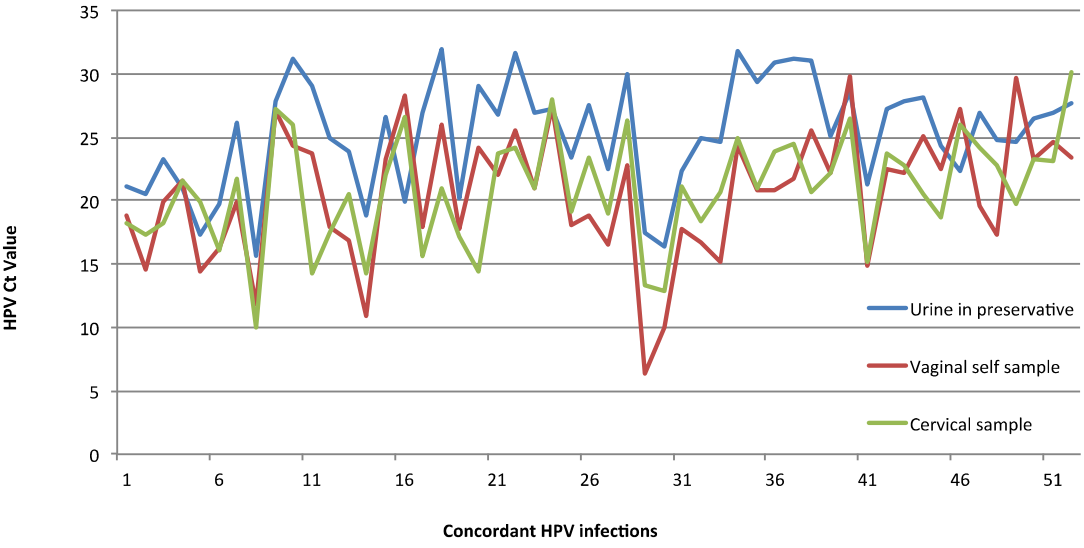

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Figure 2c

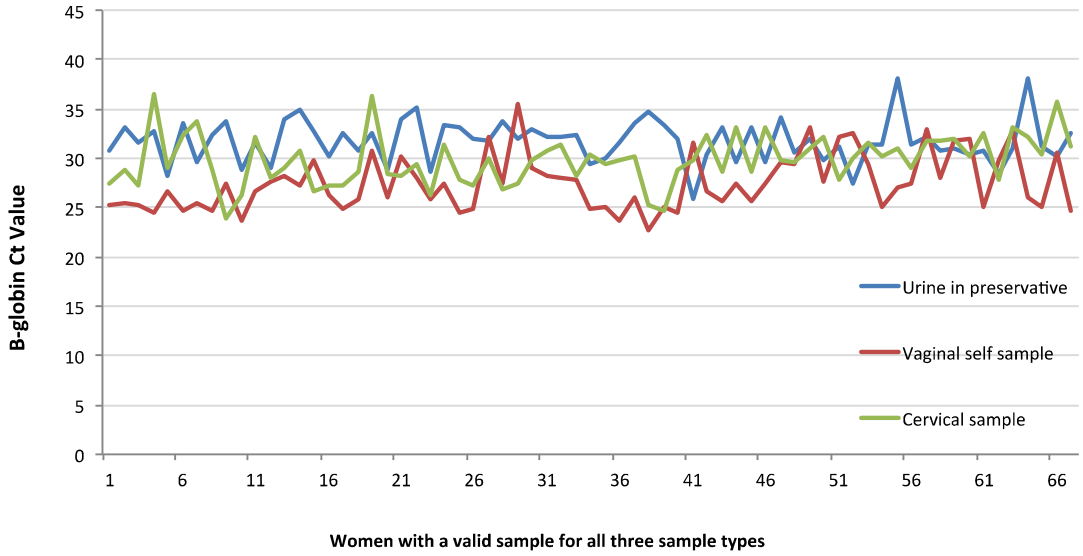

Figure 2d

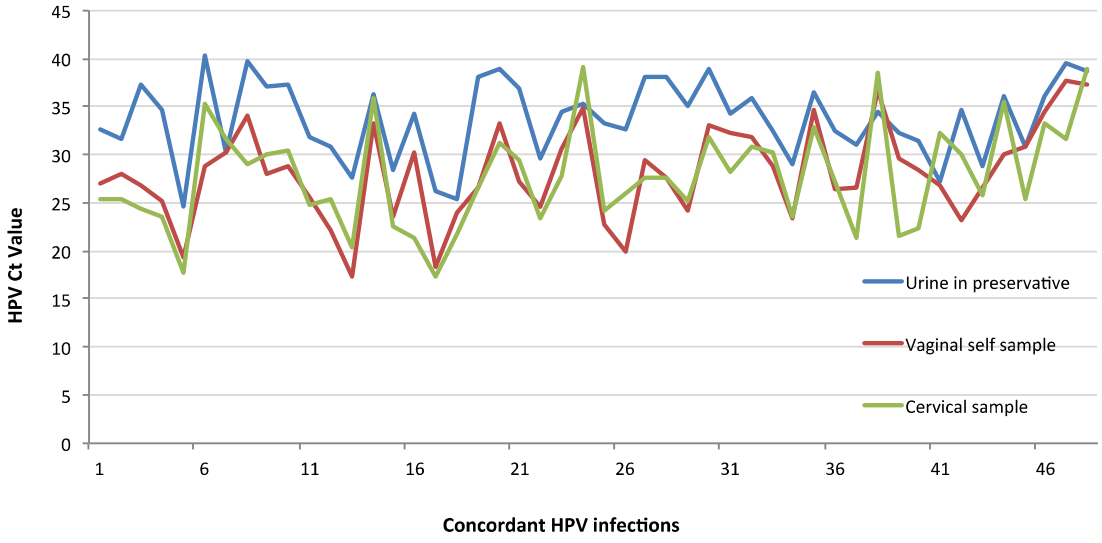

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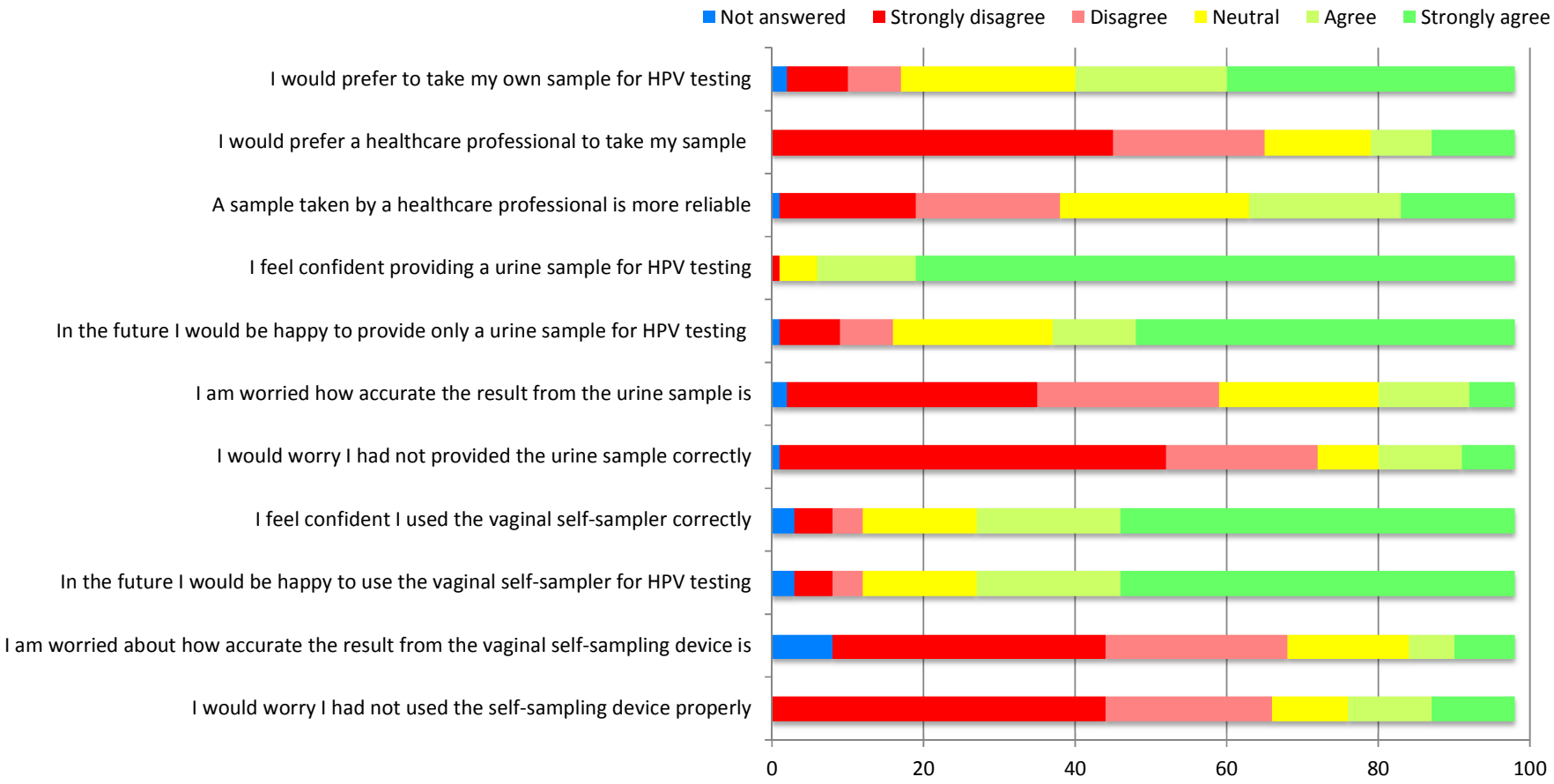
FIGURE 3: The acceptability of urine and vaginal self-obtained samples for cervical screening



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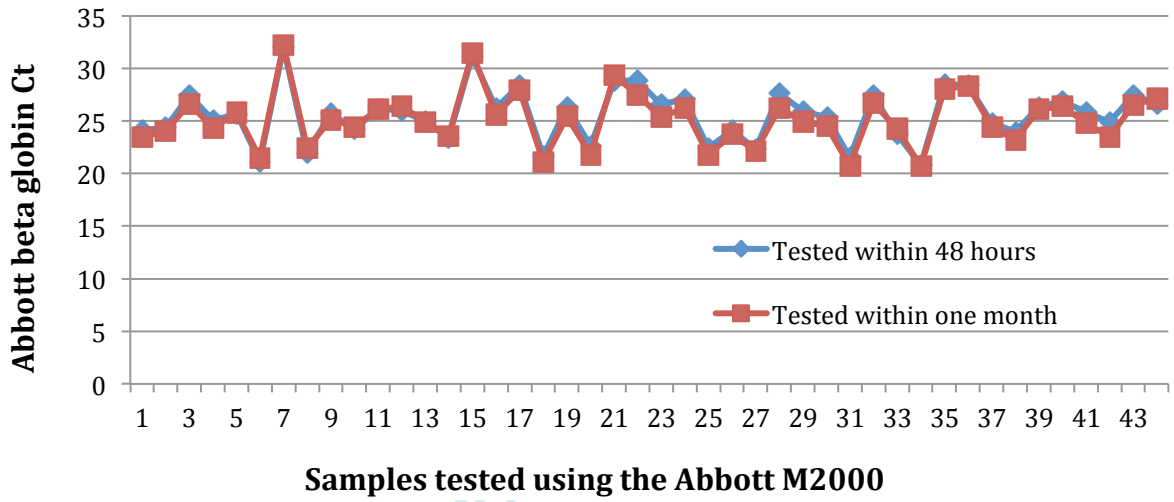
SUPPLEMENTAL FIGURE 1: Detection of internal beta globin control in preservative-fixed urine using Abbott RealTi*me* assay within 48 hours and after one month showed no reduction in DNA stability over time



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MANCHESTER 1824 Number:…… Central Manchester University Hospitals NHS NHS Foundation Trust

Urine Testing for HPV: the UVC pilot study – Questionnaire

Version One, DATE

Before completing this questionnaire please make sure you have read the information sheet. By completing this questionnaire you consent to take part in the study. To complete the questionnaire please circle the answer most applicable to you in each question, tick the correct box or write in the space provided.

Previous Knowledge of HPV

Before today had you previously heard of HPV (Human Papillomavirus)? Yes No Unsure

About the tests today

Would you have liked more information about how to provide the urine sample? Yes No

Would you have liked more information about how to use the self-sampling device? Yes No

How much do you agree with these statements? Please circle the appropriate number for each statement.

	Strongly Disagree				***Strongly Agree***
I feel confident providing a urine sample to be tested for HPV.	1	2	3	4	5
I would worry I had not provided the urine sample correctly.	1	2	3	4	5
I am worried how accurate the result from the urine sample is.	1	*2*	*3*	*4*	*5*
I feel confident I used the self-sampling device correctly.	1	2	3	4	5
I would worry I had not used the self-sampling device properly.	1	2	3	4	5
I am worried about how accurate the result from the self-sampling device is.	1	2	3	4	5
I would prefer a health professional to take my sample than to take a sample myself.	1	2	3	4	5
A sample taken by a health professional is more reliable.	1	2	3	4	5
I would prefer to take my own sample.					
In the future I would be happy to use only a urine sample to be tested for HPV.	1	2	3	4	5
In the future I would be happy to be tested for HPV from a sample taken by myself using the vaginal HPV self-sampler.	1	2	3	4	5
I felt I understood the instructions that were given to me.	1	2	3	4	5
I was happy to provide three samples.	1	2	3	4	5

Which test would you prefer to take?

Urine test Using self –sampler Clinician taken sample

What would be your second choice?

Urine test Using self –sampler Clinician taken sample

Which of these words describes how you felt about providing a urine sample? (Please circle all that apply)

Uncomfortable It was easy Embarrassed Private Reliable Convenient

Which of these words describes how you felt about using the self-sampling test? (Please circle all that apply)

Uncomfortable It was easy Embarrassed Private Reliable Convenient

Which of these words describes how you felt about having a health professional take a sample? (Please circle all that apply)

Uncomfortable It was easy Embarrassed Private Reliable Convenient

About you

What age group do you belong to? (shown in years)

24 and under 25-29 30-34 35-39 40-44 45-49 50-54 55-59 60-64 65-69 70+

Which of the following best describes your ethnicity? Please circle your answer below

White British	Indian	Caribbean	White and Black Caribbean
White Irish	Pakistani	African	White and Black African
Any other White background	Bangladeshi	Any other Black background	White and Asian
Chinese	Any other Asian background		Any other mixed background

Any Other Ethnic Group (please provide details) …….. Prefer not to say

How would you best describe your employment status?

Employed Unemployed Student Full time parent Retired

Which of these qualifications do you have? Please circle all that apply

GCE O-level/GCSE A-level or equivalent Undergraduate Postgraduate

Other (please specify) …….

Thank you for taking the time to complete this questionnaire. Please place it in the white envelope given to you and put it in the box marked 'Questionnaires' or hand it in the envelope to the clinic receptionist.

BMJ Open

A cross-sectional study of HPV testing in self-sampled urine and comparison with matched vaginal and cervical samples in women attending colposcopy for the management of abnormal cervical screening

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-025388.R1
Article Type:	Research
Date Submitted by the Author:	15-Nov-2018
Complete List of Authors:	Sargent, Alex; Manchester University NHS Foundation Trust, Clinical Virology Department Fletcher, Samantha; University of Manchester Faculty of Biology, Medicine and Health, Gynaecological Oncology Bray, Katarina; University of Manchester, Oxford Road, M13 9PL, Manchester, UK, Division of Cancer Sciences Kitchener, Henry; University of Manchester, Oxford Road, M13 9PL, Manchester, UK, Division of Cancer Sciences Crosbie, Emma; University of Manchester Faculty of Biology, Medicine and Health, Gynaecological Oncology
Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Oncology
Keywords:	Urinary human papillomavirus (HPV) detection, Cervical intraepithelial neoplasia (CIN), Cervical cancer screening, Acceptability

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A cross-sectional study of HPV testing in self-sampled urine and comparison with matched vaginal and cervical samples in women attending colposcopy for the management of abnormal cervical screening

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ABSTRACT

Objectives: Human papillomavirus (HPV) testing in cervical screening offers the potential for self-sampling to improve uptake amongst non-attenders. High-risk (HR) HPV detection in urine shows promise, but few studies have examined its sensitivity for CIN2+ detection compared with standard cervical samples. The aims of this cross-sectional study were to optimize conditions for urine testing for HPV detection; to determine concordance for HR-HPV detection in matched urine, vaginal and cervical samples; to compare the sensitivity of HR-HPV testing for the detection of CIN2+ in matched samples; and to determine the acceptability of urine testing for cervical screening.

Design: Cross-sectional study

Setting: Secondary care colposcopy clinic in North West England

Participants: Women aged 25 years of age or older, attending colposcopy clinic for management of abnormal cervical screening results or a suspicious-looking cervix. In total, 104 women took part in the study. Triple matched samples were available for 79 and 66 women using Abbott RealTi*m*e (ART) and Roche Cobas 4800 (RC) respectively.

Intervention: Self-collected urine and vaginal samples and practitioner-obtained cervical samples were tested for HR-HPV by ART and RC assays, including comparison of neat and preservative-fixed urine. Colposcopic opinion was recorded and directed cervical biopsies taken if clinically indicated. The acceptability of self-testing was evaluated by questionnaire.

Primary outcome measure: The sensitivity of urine to detect underlying CIN2+

Secondary Outcome Measures: The comparative sensitivity of vaginal and cervical samples to detect CIN2+; the acceptability of urine sampling.

Results: Preservative-fixed, but not neat urine, showed good concordance with vaginal samples for the detection of HR-HPV. The sensitivity for detecting CIN2+ was 15/18 (83%) for urine and 16/18 (89%) for cervical and vaginal samples by ART, and 15/17 (88%) for all samples by RC. Urine-based testing was broadly acceptable to women.

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Conclusions: Urinary HR-HPV detection offers an alternative strategy of cervical screening. Larger studies to determine its clinical utility are warranted.

Keywords: Urinary human papillomavirus (HPV) detection, cervical intraepithelial neoplasia (CIN), cervical cancer screening, acceptability

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Strengths and limitations of this study

- We studied the clinical performance of urine HPV testing in colposcopy clinic attendees, not cervical screening non-attenders, in whom the technology is likely to have most value
- We compared neat and preservative-fixed urine using two different HR-HPV detection assays alongside matched vaginal and cervical samples
- The virologist was blinded to clinical outcomes at the time of HR-HPV testing
- The clinical performance of urine HPV testing may be affected by the prevalence of HR-HPV in the population, which is higher in colposcopy clinic attendees than cervical screening non-attenders
- We determined the acceptability of urine HPV testing by studying women's preferences for urine, vaginal and cervical sampling, whilst acknowledging that cervical screening attenders may have different preferences to non-attenders

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BACKGROUND

The strategy of cervical screening depends on reducing the incidence of cervical cancer through the detection and treatment of its precursor lesion, high grade cervical intraepithelial neoplasia (CIN2+) (1). Cervical cancer is caused by persistent infection with high-risk human papillomavirus (HR-HPV) (2) and a pooled analysis of four large RCTS found that detection of HR-HPV in practitioner-obtained cervical samples is a more sensitive primary screen than cervical cytology for the detection of CIN2+ (3). The UK National Screening Committee (UK NSC), together with other publically funded programs has therefore recommended moving to primary HPV testing for general population screening, with reflex cytology on positive samples.

Despite the success of cervical screening, recent years have witnessed a gradual decline in its uptake, particularly by women aged 25-49 years. Barriers to cervical screening include embarrassment, fear, inconvenience and some women believe that they are not at risk (4). It may be possible to overcome some of these barriers by improving the acceptability of the screening test and the ease with which it can be performed. Vaginal self-sampling kits for HR-HPV detection have a modest impact on screening uptake by chronic non-attenders (5, 6). The diagnostic accuracy of HPV detection in self-obtained vaginal samples, while less than for practitioner-obtained cervical samples, is sufficient to recommend its use in non-attenders and in low resource settings (7).

HPV detection in urine has recently been considered as an alternative means to identify women at increased risk of high grade CIN. A systematic review with meta-analysis found a pooled sensitivity of 77% and specificity of 88% for HR-HPV detection in urine (8, 9). This pooled sensitivity was lower than for HR-HPV detection in practitioner-obtained cervical samples but the wide variation in methodology, including urine sampling techniques, HPV detection methodology and study setting precluded unbiased comparison. Since HPV testing is more sensitive than cytology for the detection

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of CIN2+, a degree of decreased sensitivity of urinary HPV detection could be offset by improved uptake of cervical screening (10).

The aims of this exploratory study were fourfold: to optimize urine processing for HR-HPV detection; to establish the concordance of HR-HPV detection in matched urine, vaginal and cervical samples in a colposcopy population; to compare the sensitivity of HR-HPV testing for CIN2+ detection in the same samples; and to determine the acceptability by women of self-collected versus practitioner-obtained samples for cervical screening.

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METHODS

Study participants and sample collection

Urine, vaginal and cervical samples were collected from consecutive women attending the colposcopy clinic at St Mary's Hospital, Manchester University Foundation Trust (MFT) between June and September 2015. A participant information sheet was sent with the colposcopy appointment letter to women who were 25 years of age or older and attending the colposcopy clinic for management of abnormal cervical screening results or a suspicious-looking cervix. The study was approved by the North West Research Ethics Committee (15/NW/0218) and all women gave written, informed consent to take part. Women who declined participation in the study were asked to give their reasons. Pregnant women were excluded from the study. Study samples were obtained prior to colposcopic examination of the cervix, where this was required. Self-collected first stream urine samples were collected in a sterile dry pot, followed by self-collected vaginal samples obtained using the Rovers Evalyn Brush (Netherlands). Women were asked to place the brush in the vagina, rotate it five times for sample collection and replace the brush in its casing. Practitioner-obtained cervical samples were then taken with a cervical broom immediately prior to clinically-indicated procedures. Following colposcopy, women were asked to complete a questionnaire about the acceptability of the tests performed (**Supplemental Appendix 1**).

Clinical procedures

Women were managed clinically according to NHS Cervical Screening Programme guidelines. All colposcopists were accredited for independent practice by the British Society of Colposcopy and Cervical Pathology (BSCCP). Abnormal colposcopy indicated directed punch biopsy and histological review by consultant gynecological histopathologists, who classified biopsies as normal, low grade CIN (CIN1), or high grade CIN (CIN2/3).

HPV DNA testing

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Blinded samples were delivered to the MFT Virology laboratory within four hours of collection. Cervical samples were collected using SurePath Liquid Based Cytology (LBC) and vaginal cells were suspended in solution by agitating the Evalyn brush in SurePath preservative on receipt in the laboratory. Sample aliquots were added to Abbott and Roche secondary tubes as per the manufacturers' instructions. Urine samples were refrigerated at 4 degrees both without preservative ('neat') and following transfer to Abbott and Roche preservative tubes ('preservative-fixed') using the Abbott multi-collect specimen collection kit and the Roche Cobas PCR urine specimen collection kit. Batches of samples were tested for high risk HPV using the Abbott RealTi*m*e (ART) and Roche Cobas 4800 (RC) assays within 48 hours and one week of sample processing, respectively. Both assays used fully automated systems that amplify and detect 14 HR-HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) simultaneously. Separate probes enable the specific detection of HPV types 16 and 18. Both assays also measure human beta globin as a control for sample cellularity, valid sample extraction and amplification. The cycle threshold (ct) is a measure of DNA concentration, with ct values inversely proportional to the amount of target nucleic acid in the sample. All samples were tested according to the manufacturers' instructions. Both assays (ART and RC) were applied to all samples, where sufficient material was available.

Statistical analysis

HPV detection rates were calculated for each assay and sample type. Concordance between the different samples for HR-HPV positivity was determined using Cohen's kappa statistic. Agreement was slight (k<0.20), weak (k=0.21-0.40), moderate (k=0.41-0.60), substantial (k=0.61-0.80), near perfect (k=0.81-0.99), or perfect (k=1.00). The sensitivity of HR-HPV positivity for CIN2+ detection was compared between urine, vaginal and cervical samples. Sensitivity was calculated as the number of true positives divided by the sum of the number of true positives plus false negatives.

Patient and public involvement

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Patients and the public were not involved in designing or conducting this research. We will work with patient support groups and forums, including Jo's Cervical Cancer Trust, to help disseminate the results of this study.

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RESULTS

Study participants

One hundred and four eligible women took part in the study (**Figure 1**). Their median age range and distribution reflected that of routine colposcopic practice, with 40% between 25 and 29 years of age. Non-participants had similar demographics to those who did agree to the study. The reasons for non-participation are shown in **Figure 1**. Triple matched samples were not available for 17 women (no vaginal sample n=8; no cervical sample n=5; no urine sample n=1; only urine sample n=2; only cervical sample n=1).

Optimization of urine sample processing for HR-HPV testing

A comparison was made between neat and preservative-fixed urine for HR-HPV detection using both ART and RC assays for the first 50 study participants. There were 44 matched neat and preservative-fixed urine sample pairs available for the ART assay. Two failed the internal beta globin control and were excluded from further analysis. A cycle threshold (ct) cut off of ≤32 was used to define HR-HPV positivity. **Table 1** demonstrates good concordance between neat and preservative-fixed urine samples (37/42, 88%, k=0.76, 95%CI 0.56-0.96).

TABLE 1: Comparison of neat versus preservative-fixed urine using the Abbott RealTi*m*e (ART) assay and the Roche Cobas 4800 (RC) assay for HR-HPV detection

ART assay	**Neat positive**	**Neat negative**	**Total**
Preservative positive	19	3	22
Preservative negative	2	18	20
Total	21	21	42
RC assay	**Neat positive**	**Neat negative**	**Total**
Preservative positive	14	2	16
Preservative negative	0	6	6

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Total	14	8	22

Repeat testing of the 47 preservative-fixed urine samples approximately one month later (mean 33 days) showed no reduction in stability of HR-HPV DNA, with almost identical ct values, as shown in **Supplemental Figure 1** (mean ct 25.2 for samples tested within 48 hours versus 25.1 for samples tested at one month). This was true when all HR-HPV types, HPV 16/18 and HR-HPV other were considered separately (data not shown).

There were 40 matched neat and preservative-fixed urine sample pairs available for the RC assay. Eighteen of the neat but none of the preservative-fixed urine samples, which had been stored for up to one week prior to testing, failed the internal beta globin control and were excluded from further analysis. Thus there were 22 matched urine sample pairs available for testing with the RC assay. A cycle threshold of ≤40 was used to define HR-HPV positivity. **Table 1** shows good concordance between neat and preservative-fixed urine samples (20/22, 90%, k=0.79, 95%CI 0.52-1.0). Based on these data and the apparent instability of neat urine samples over time, all subsequent urine samples were fixed with preservative upon receipt in the lab, and neat urine samples were not considered further.

HR-HPV detection in urine, vaginal and cervical samples

Abbott RealTi*m*e assay

From the 104 women, 79 had valid HR-HPV results from matched urine, vaginal and cervical samples for the ART assay. Triple samples were not available for 17 women and invalid results were obtained for one or more sample for a further 8 women. There were no invalid results for urine samples. Directed cervical biopsies were taken from 66/79 (84%) of these women because of abnormal

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cytology. Histological analysis classified the biopsies within normal limits (31/66, 47%), CIN1 (17/66, 26%), CIN2 (10/66, 15%) and CIN3 (8/66, 12%).

HR-HPV was detected in 48/79 (61%), 54/79 (68%) and 56/79 (71%) of urine, vaginal and cervical samples respectively. HPV 16/18 was detected in 21/79 (27%), 24/79 (30%) and 23/79 (29%). Concordance between the three sample types was found in 62/79 (79%), as shown in **Table 2**. According to the manufacturer's cut-off of 32ct, the sensitivity for detecting CIN2+ was 16/18 (89%) for both cervical and vaginal samples and 15/18 (83%) for urine samples. Concordance between urine and cervical samples was 82% (65/79; k=0.61; 95% CI 0.43-0.79) and between vaginal and cervical samples was 90% (71/79; k=0.76; 95% CI 0.61-0.92). Only one CIN2+ lesion was 'missed' from the 8 women with invalid results by the ART assay.

TABLE 2: HR-HPV detection in preservative-fixed urine, vaginal and cervical samples by the Abbott RealTi*m*e (ART) assay and the Roche Cobas 4800 (RC) assay

		Cervical positive		Cervical negative	
		Vaginal positive	**Vaginal negative**	**Vaginal positive**	**Vaginal negative**
ART	**Urine positive**	43[a]	2	2	1
	Urine negative	8[b]	3	1	19[c]
RC					
	Urine positive	44[d]	0	1	0
	Urine negative	4	0	1	16[e]

[a]9 CIN2, 6 CIN3; [b]1 CIN3; [c]1 CIN2, 1 CIN3; [d]8 CIN2, 7 CIN3; [e]1 CIN2, 1 CIN3

A comparison of the ct values for detection of the internal beta globin control demonstrated lower DNA concentrations in urine compared with vaginal and cervical samples (**Figure 2a**). The mean ct value for urine, vaginal and cervical samples was 25.6, 20.9 and 23.1 respectively, with similar results

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for samples which showed positive results for HPV 16/18 or HPV other (25.3, 20.6 and 20.9 respectively) (**Figure 2b**).

Roche Cobas 4800 assay

From the 104 women, 66 had valid HR-HPV results from matched urine, vaginal and cervical samples for the RC assay. Triple samples were not available for 17 women, there was insufficient volume for the RC assay for 8 cervical and 3 urine samples, and invalid results were obtained for one or more sample for 10 women. There were no invalid results for urine samples. Cervical biopsies were taken for 54/66 (82%) of these women and histological analysis classified these as normal (24/54, 44%), CIN1 (14/54, 26%), CIN2 (8/54, 15%) and CIN3 (8/54, 15%).

HR-HPV was detected in 45/66 (68%), 50/66 (76%) and 48/66 (73%) of urine, vaginal and cervical samples respectively. HPV 16/18 was detected in 16/66 (24%), 21/66 (32%) and 19/66 (29%). Concordance between the three sample types was found in 60/66 (91%), as shown in **Table 2**. According to the manufacturer's cut-off of 40ct, the sensitivity for detecting CIN2+ was 15/17 (88%) for all three sample types. Concordance between urine and cervical samples was 92% (61/66, k=0.82, 95% CI 0.67–0.97) and between vaginal and cervical samples was 97% (64/66, k=0.77, 95% CI 0.60-0.92). No CIN2+ lesions were 'missed' amongst women with invalid results by the RC assay.

A comparison of the mean ct values for detection of the internal beta globin control again demonstrated lower DNA concentrations in urine (**Figure 2c**). The mean ct values for urine, vaginal and cervical samples were 31.7, 27.5 and 29.9 respectively, with similar findings with concordant positive results for HPV 16/18 or HPV other (33.6, 28.1 and 27.7 respectively) (**Figure 2d**).

Acceptability of urine, vaginal and cervical sampling for cervical screening

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A total of 98 women answered the acceptability questionnaire (**Supplemental Appendix 1**). Most of the participants had heard about HPV before (yes, 73; no, 17; don't know, 1; not answered, 7). Participants were confident about providing self-obtained vaginal and urine samples for HPV testing and were not excessively concerned about the accuracy of the test in these samples (**Figure 3**). In general, women were more confident about providing a urine sample than a vaginal sample for HPV testing. When asked which sample they would prefer to provide, more women chose urine (39) than either vaginal (32) or cervical samples (17; no preference, 1; not answered, 9).

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DISCUSSION

This study found good concordance for HR-HPV detection between preservative-fixed urine, vaginal and cervical samples amongst women attending a colposcopy clinic. Urine showed similar, albeit slightly inferior, sensitivity to matched vaginal and cervical samples for CIN2+ detection using both ART and RC assays. Cervical screening by urinary HR-HPV detection was considered acceptable by women.

This was an exploratory study of women attending the colposcopy clinic for the management of abnormal cervical screening results or a suspicious-looking cervix. Forty percent of the participants were under 30 years of age. The prevalence of HR-HPV was therefore sufficiently high to allow comparison between the three sampling methods. Matched urine, vaginal and cervical samples taken at the same visit and immediately prior to colposcopic assessment, eliminated the possibility that HPV had been acquired or cleared between tests. The order of obtaining matched samples meant that cross-contamination was unlikely. We found that preservative-fixed urine was suitable for HR-HPV detection by both ART and RC assays within 48 hours and up to one month following its receipt. The consistent results showed that preservative-fixed urine can be handled in the same way as cervical samples without compromising analytical sensitivity.

The relatively small size of the study means that urine testing needs to be fully validated in larger cohorts. The colposcopy clinic setting facilitated an assessment of the analytical sensitivity of urinary HR-HPV testing but the true value of urine-based cervical screening is likely to be its ability to improve cervical screening uptake by non-attenders. The prevalence of HR-HPV is much lower in the general population, which could be expected to affect the sensitivity and negative predictive value of the test. Studies designed to evaluate the accuracy and clinical utility of urinary HR-HPV detection in the primary cervical screening population are therefore required (11). Urinary self-sampling was

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acceptable to women attending colposcopy clinic; those who declined participation did so for other reasons. An important limitation of this study is that self-sampling took place before colposcopy, which may have affected women's confidence in the accuracy of self-sampling. The acceptability of urinary self-sampling to cervical screening non-attenders remains unclear, as does the ability of urinary HR-HPV detection to improve uptake. Defining its acceptability in 'hard to reach' groups is challenging but crucial to its further development as an alternative test for cervical screening. Forty percent of our participants were under 30 years of age, a group of women that stands to benefit most from strategies to improve uptake, given their declining participation in cervical screening programmes (5).

Previous studies have looked at urine sampling techniques (12, 13) but few have reported the optimization of urine processing and storage for HR-HPV detection. Here we show that preservative-fixed urine has improved stability and can be stored prior to testing for much longer than neat urine, which rapidly deteriorates after 48 hours. This may explain the improved clinical performance of urinary HR-HPV reported here compared to studies that tested neat urine (8). In total, 45% of neat urine samples tested on the RC assay at one week were invalid compared to only 2% of samples tested within 48 hours of collection by ART assay. This can be explained by an increase in PCR inhibitors over time. Preservative-fixed urine stored at 4^{o}C showed only one invalid result using the ART assay amongst 104 urine samples tested within 48 hours, and there were no invalid results using the RC assay amongst 98 urine samples tested between 7 and 14 days. Repeat testing at one month showed consistent results. Whilst the requirement to preserve urine samples challenges the ideal scenario of home collection and postage of urine samples to the laboratory, it would not preclude self-collection within Primary Care, where preservative can be safely added shortly afterwards.

The analytical and clinical sensitivity of the RC and ART assays for cervical HR-HPV detection are well documented (14-16), and both assays are approved for primary HPV screening, HPV triage of minor

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cytological abnormalities and test of cure in the NHS Cervical Screening Programme. Neither assay is currently CE marked for urinary testing and whilst the data in this pilot study are consistent with published studies, more work is needed to define viral load thresholds of clinical utility for the detection of CIN2+ (17, 18). Raising the threshold of the ART assay above 32ct (manufacturer's cut-off) did pick up four more positive urine samples but did not identify the single undetected CIN2+, which suggests that the manufacturer's cut-offs are appropriate for urine samples. The analytical sensitivity for HR-HPV in urine, vaginal and cervical samples were similar though a lower proportion of HR-HPV was detected in urine samples compared with vaginal samples.

Barriers to cervical screening include embarrassment, fear, inconvenience and the view held by some women that they are not at risk (4). Offering an alternative means of cervical screening can encourage non-attenders to be screened, either by 'nudging' them to undergo cervical sampling or by facilitating self-sampling at home (5). Urine-based testing is already widely used for the detection of chlamydia and is generally trusted and acceptable to women. The disadvantage of self-testing is that reflex cytology is not possible in the event of a positive HR-HPV test, however, data suggest that women who screen positive for HR-HPV are highly likely to attend for cervical cytology (19). Urine-based testing may also be cheaper than standard cervical screening tests because it does not require an appointment, a practitioner or any special equipment and it lends itself to high throughput testing. Thus it could also be suitable in resource poor settings where the infrastructure required to underpin screening by cervical cytology is lacking.

CONCLUSIONS

Urinary HR-HPV testing has good sensitivity for the detection of CIN2+ in a colposcopy clinic cohort. Our study suggests that vaginal HR-HPV testing is slightly more sensitive than urine HR-HPV testing, however urine self-sampling may be more acceptable to cervical screening non-attenders than vaginal self-sampling. Future studies are warranted to assess the performance and acceptability of

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urine self-sampling in comparison with vaginal self-sampling in the general screening population.

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LIST OF ABBREVIATIONS

ART	Abbott RealTi*m*e
BSCCP	British Society of Colposcopy and Cervical Pathology
CIN	cervical intraepithelial neoplasia
ct	cycle threshold
HR	high risk
HPV	human papillomavirus
LBC	liquid based cytology
PCR	polymerase chain reaction
RC	Roche Cobas 4800
UK NSC	UK National Screening Committee

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DECLARATIONS

Ethics approval and consent to participate

The study was approved by the North West Research Ethics Committee (15/NW/0218) and all women gave written, informed consent to take part.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

HK is Chair of the Advisory Committee for Cervical Screening (PHE), but the views expressed in this manuscript are those of the author and not Public Health England.

Funding

EC is a National Institute for Health Research (NIHR) Clinician Scientist (NIHR-CS-012-009). This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Author contributions

AS performed the laboratory analyses, data interpretation and wrote the first draft of the manuscript. SF and KB recruited and sampled the study participants and contributed to data interpretation. HK and EC designed the study and contributed to data interpretation and manuscript preparation. All authors reviewed the final version of the manuscript.

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Acknowledgements

The authors would like to thank all the women who participated in this study and donated their samples and data for research. We would like to thank Mrs. Linsey Nelson for her support with obtaining all the necessary regulatory approvals and for data management.

Data sharing statement

Data are available and will be shared upon reasonable request.

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FIGURE 1: Demographics of study participants and reasons for declining participation. Key: PG, postgraduate; UG, undergraduate

FIGURE 2: A comparison of the mean ct values for detection of the internal beta globin control and HR-HPV in urine, vaginal and cervical samples using the ART (a, b) and RC assays (c, d).

FIGURE 3: The acceptability of urine and vaginal self-obtained samples for cervical screening

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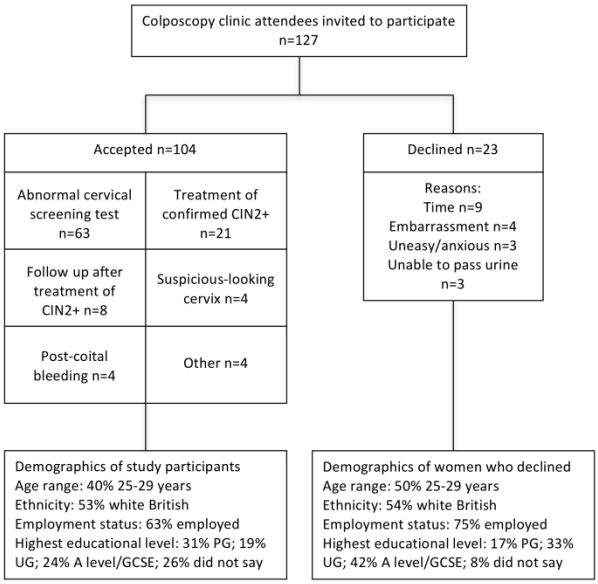

FIGURE 1: Demographics of study participants and reasons for declining participation. Key: PG, postgraduate; UG, undergraduate

209x296mm (300 x 300 DPI)

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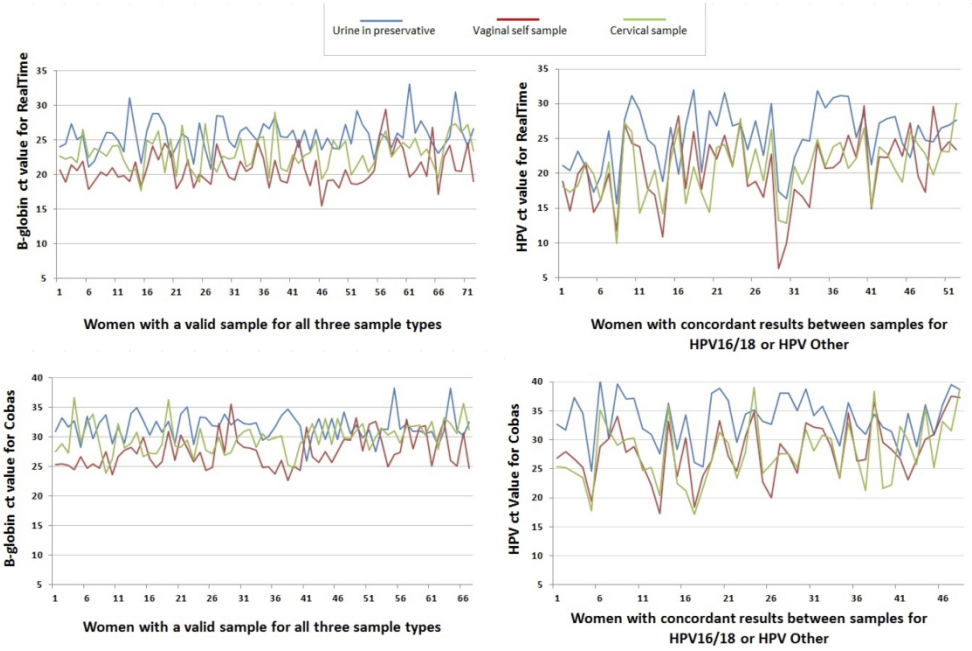

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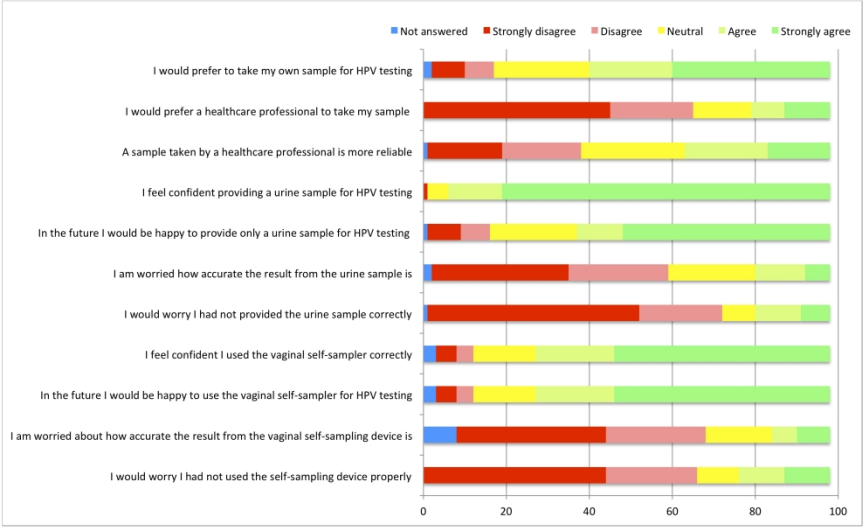

FIGURE 3: The acceptability of urine and vaginal self-obtained samples for cervical screening

297x209mm (300 x 300 DPI)

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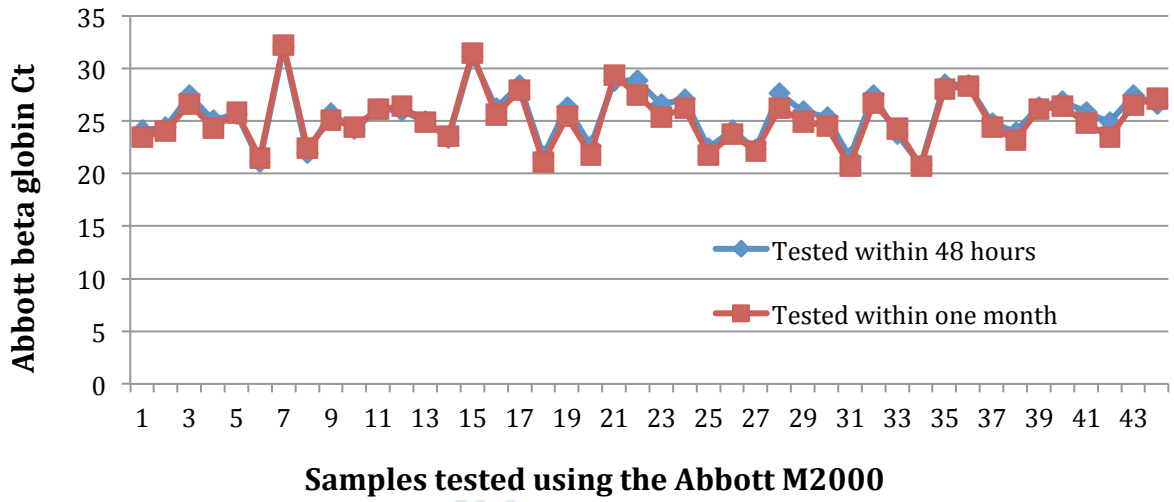
SUPPLEMENTAL FIGURE 1: Detection of internal beta globin control in preservative-fixed urine using Abbott RealTi*me* assay within 48 hours and after one month showed no reduction in DNA stability over time



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MANCHESTER 1824 Number:…… Central Manchester University Hospitals NHS NHS Foundation Trust

Urine Testing for HPV: the UVC pilot study – Questionnaire

Version One, DATE

Before completing this questionnaire please make sure you have read the information sheet. By completing this questionnaire you consent to take part in the study. To complete the questionnaire please circle the answer most applicable to you in each question, tick the correct box or write in the space provided.

Previous Knowledge of HPV

Before today had you previously heard of HPV (Human Papillomavirus)? Yes No Unsure

About the tests today

Would you have liked more information about how to provide the urine sample? Yes No

Would you have liked more information about how to use the self-sampling device? Yes No

How much do you agree with these statements? Please circle the appropriate number for each statement.

	Strongly Disagree				***Strongly Agree***
I feel confident providing a urine sample to be tested for HPV.	1	2	3	4	5
I would worry I had not provided the urine sample correctly.	1	2	3	4	5
I am worried how accurate the result from the urine sample is.	1	*2*	*3*	*4*	*5*
I feel confident I used the self-sampling device correctly.	1	2	3	4	5
I would worry I had not used the self-sampling device properly.	1	2	3	4	5
I am worried about how accurate the result from the self-sampling device is.	1	2	3	4	5
I would prefer a health professional to take my sample than to take a sample myself.	1	2	3	4	5
A sample taken by a health professional is more reliable.	1	2	3	4	5
I would prefer to take my own sample.					
In the future I would be happy to use only a urine sample to be tested for HPV.	1	2	3	4	5
In the future I would be happy to be tested for HPV from a sample taken by myself using the vaginal HPV self-sampler.	1	2	3	4	5
I felt I understood the instructions that were given to me.	1	2	3	4	5
I was happy to provide three samples.	1	2	3	4	5

Which test would you prefer to take?

Urine test Using self –sampler Clinician taken sample

What would be your second choice?

Urine test Using self –sampler Clinician taken sample

Which of these words describes how you felt about providing a urine sample? (Please circle all that apply)

Uncomfortable It was easy Embarrassed Private Reliable Convenient

Which of these words describes how you felt about using the self-sampling test? (Please circle all that apply)

Uncomfortable It was easy Embarrassed Private Reliable Convenient

Which of these words describes how you felt about having a health professional take a sample? (Please circle all that apply)

Uncomfortable It was easy Embarrassed Private Reliable Convenient

About you

What age group do you belong to? (shown in years)

24 and under 25-29 30-34 35-39 40-44 45-49 50-54 55-59 60-64 65-69 70+

Which of the following best describes your ethnicity? Please circle your answer below

White British	Indian	Caribbean	White and Black Caribbean
White Irish	Pakistani	African	White and Black African
Any other White background	Bangladeshi	Any other Black background	White and Asian
Chinese	Any other Asian background		Any other mixed background

Any Other Ethnic Group (please provide details) …….. Prefer not to say

How would you best describe your employment status?

Employed Unemployed Student Full time parent Retired

Which of these qualifications do you have? Please circle all that apply

GCE O-level/GCSE A-level or equivalent Undergraduate Postgraduate

Other (please specify) …….

Thank you for taking the time to complete this questionnaire. Please place it in the white envelope given to you and put it in the box marked 'Questionnaires' or hand it in the envelope to the clinic receptionist.

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	2
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	5
	4	Study objectives and hypotheses	6
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	7
Participants	**6**	Eligibility criteria	7
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	7
	8	Where and when potentially eligible participants were identified (setting, location and dates)	7
	9	Whether participants formed a consecutive, random or convenience series	7
Test methods	**10a**	Index test, in sufficient detail to allow replication	7
	10b	Reference standard, in sufficient detail to allow replication	7
	11	Rationale for choosing the reference standard (if alternatives exist)	7
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	8
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	8
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	8
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	7
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	8
	15	How indeterminate index test or reference standard results were handled	
	16	How missing data on the index test and reference standard were handled	
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	
	18	Intended sample size and how it was determined	
RESULTS			
Participants	**19**	Flow of participants, using a diagram	Figure 1
	20	Baseline demographic and clinical characteristics of participants	Figure 1
	21a	Distribution of severity of disease in those with the target condition	11, 13
	21b	Distribution of alternative diagnoses in those without the target condition	Figure 1
	22	Time interval and any clinical interventions between index test and reference standard	7
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	12
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	10-13
	25	Any adverse events from performing the index test or the reference standard	
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	15, 16
	27	Implications for practice, including the intended use and clinical role of the index test	15, 16, 17
OTHER INFORMATION			
	28	Registration number and name of registry	7
	29	Where the full study protocol can be accessed	
	30	Sources of funding and other support; role of funders	19

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STARD 2015

AIM

STARD stands for "Standards for Reporting Diagnostic accuracy studies". This list of items was developed to contribute to the completeness and transparency of reporting of diagnostic accuracy studies. Authors can use the list to write informative study reports. Editors and peer-reviewers can use it to evaluate whether the information has been included in manuscripts submitted for publication.

EXPLANATION

A **diagnostic accuracy study** evaluates the ability of one or more medical tests to correctly classify study participants as having a **target condition.** This can be a disease, a disease stage, response or benefit from therapy, or an event or condition in the future. A medical test can be an imaging procedure, a laboratory test, elements from history and physical examination, a combination of these, or any other method for collecting information about the current health status of a patient.

The test whose accuracy is evaluated is called **index test.** A study can evaluate the accuracy of one or more index tests. Evaluating the ability of a medical test to correctly classify patients is typically done by comparing the distribution of the index test results with those of the **reference standard**. The reference standard is the best available method for establishing the presence or absence of the target condition. An accuracy study can rely on one or more reference standards.

If test results are categorized as either positive or negative, the cross tabulation of the index test results against those of the reference standard can be used to estimate the **sensitivity** of the index test (the proportion of participants *with* the target condition who have a positive index test), and its **specificity** (the proportion *without* the target condition who have a negative index test). From this cross tabulation (sometimes referred to as the contingency or "2x2" table), several other accuracy statistics can be estimated, such as the positive and negative **predictive values** of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical **precision** of the measurements.

If the index test results can take more than two values, categorization of test results as positive or negative requires a **test positivity cut-off**. When multiple such cut-offs can be defined, authors can report a receiver operating characteristic (ROC) curve which graphically represents the combination of sensitivity and specificity for each possible test positivity cut-off. The **area under the ROC curve** informs in a single numerical value about the overall diagnostic accuracy of the index test.

The **intended use** of a medical test can be diagnosis, screening, staging, monitoring, surveillance, prediction or prognosis. The **clinical role** of a test explains its position relative to existing tests in the clinical pathway. A replacement test, for example, replaces an existing test. A triage test is used before an existing test; an add-on test is used after an existing test.

Besides diagnostic accuracy, several other outcomes and statistics may be relevant in the evaluation of medical tests. Medical tests can also be used to classify patients for purposes other than diagnosis, such as staging or prognosis. The STARD list was not explicitly developed for these other outcomes, statistics, and study types, although most STARD items would still apply.

DEVELOPMENT

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on http://www.equator-network.org/reporting-guidelines/stard.

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A cross-sectional study of HPV testing in self-sampled urine and comparison with matched vaginal and cervical samples in women attending colposcopy for the management of abnormal cervical screening

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-025388.R2
Article Type:	Research
Date Submitted by the Author:	03-Jan-2019
Complete List of Authors:	Sargent, Alex; Manchester University NHS Foundation Trust, Clinical Virology Department Fletcher, Samantha; University of Manchester Faculty of Biology, Medicine and Health, Gynaecological Oncology Bray, Katarina; University of Manchester, Oxford Road, M13 9PL, Manchester, UK, Division of Cancer Sciences Kitchener, Henry; University of Manchester, Oxford Road, M13 9PL, Manchester, UK, Division of Cancer Sciences Crosbie, Emma; University of Manchester Faculty of Biology, Medicine and Health, Gynaecological Oncology
Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Oncology
Keywords:	Urinary human papillomavirus (HPV) detection, Cervical intraepithelial neoplasia (CIN), Cervical cancer screening, Acceptability

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A cross-sectional study of HPV testing in self-sampled urine and comparison with matched vaginal and cervical samples in women attending colposcopy for the management of abnormal cervical screening

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ABSTRACT

Objectives: Human papillomavirus (HPV) testing in cervical screening offers the potential for self-sampling to improve uptake amongst non-attenders. High-risk (HR) HPV detection in urine shows promise, but few studies have examined its sensitivity for CIN2+ detection compared with standard cervical samples. The aims of this cross-sectional study were to optimize conditions for urine testing for HPV detection; to determine concordance for HR-HPV detection in matched urine, vaginal and cervical samples; to compare the sensitivity of HR-HPV testing for the detection of CIN2+ in matched samples; and to determine the acceptability of urine testing for cervical screening.

Design: Cross-sectional study

Setting: Secondary care colposcopy clinic in North West England

Participants: Women aged 25 years of age or older, attending colposcopy clinic for management of abnormal cervical screening results or a suspicious-looking cervix. In total, 104 women took part in the study. Triple matched samples were available for 79 and 66 women using Abbott RealTi*m*e (ART) and Roche Cobas 4800 (RC) respectively.

Intervention: Self-collected urine and vaginal samples and practitioner-obtained cervical samples were tested for HR-HPV by ART and RC assays, including comparison of neat and preservative-fixed urine. Colposcopic opinion was recorded and directed cervical biopsies taken if clinically indicated. The acceptability of self-testing was evaluated by questionnaire.

Primary outcome measure: The sensitivity of urine to detect underlying CIN2+

Secondary Outcome Measures: The comparative sensitivity of vaginal and cervical samples to detect CIN2+; the acceptability of urine sampling.

Results: Preservative-fixed, but not neat urine, showed good concordance with vaginal samples for the detection of HR-HPV. The sensitivity for detecting CIN2+ was 15/18 (83%) for urine and 16/18 (89%) for cervical and vaginal samples by ART, and 15/17 (88%) for all samples by RC. Urine-based testing was broadly acceptable to women.

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Conclusions: Urinary HR-HPV detection offers an alternative strategy of cervical screening. Larger studies to determine its clinical utility are warranted.

Keywords: Urinary human papillomavirus (HPV) detection, cervical intraepithelial neoplasia (CIN), cervical cancer screening, acceptability

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Strengths and limitations of this study

- We studied the clinical performance of urine HPV testing in colposcopy clinic attendees, not cervical screening non-attenders, in whom the technology is likely to have most value
- We compared neat and preservative-fixed urine using two different HR-HPV detection assays alongside matched vaginal and cervical samples
- The virologist was blinded to clinical outcomes at the time of HR-HPV testing
- The clinical performance of urine HPV testing may be affected by the prevalence of HR-HPV in the population, which is higher in colposcopy clinic attendees than cervical screening non-attenders
- We determined the acceptability of urine HPV testing by studying women's preferences for urine, vaginal and cervical sampling, whilst acknowledging that cervical screening attenders may have different preferences to non-attenders

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BACKGROUND

The strategy of cervical screening depends on reducing the incidence of cervical cancer through the detection and treatment of its precursor lesion, high grade cervical intraepithelial neoplasia (CIN2+) (1). Cervical cancer is caused by persistent infection with high-risk human papillomavirus (HR-HPV) (2) and a pooled analysis of four large RCTS found that detection of HR-HPV in practitioner-obtained cervical samples is a more sensitive primary screen than cervical cytology for the detection of CIN2+ (3). The UK National Screening Committee (UK NSC), together with other publically funded programs has therefore recommended moving to primary HPV testing for general population screening, with reflex cytology on positive samples.

Despite the success of cervical screening, recent years have witnessed a gradual decline in its uptake, particularly by women aged 25-49 years. Barriers to cervical screening include embarrassment, fear, inconvenience and some women believe that they are not at risk (4). It may be possible to overcome some of these barriers by improving the acceptability of the screening test and the ease with which it can be performed. Vaginal self-sampling kits for HR-HPV detection have a modest impact on screening uptake by chronic non-attenders (5, 6). The diagnostic accuracy of HPV detection in self-obtained vaginal samples, while less than for practitioner-obtained cervical samples, is sufficient to recommend its use in non-attenders and in low resource settings (7).

HPV detection in urine has recently been considered as an alternative means to identify women at increased risk of high grade CIN. A systematic review with meta-analysis found a pooled sensitivity of 77% and specificity of 88% for HR-HPV detection in urine (8, 9). This pooled sensitivity was lower than for HR-HPV detection in practitioner-obtained cervical samples but the wide variation in methodology, including urine sampling techniques, HPV detection methodology and study setting precluded unbiased comparison. Since HPV testing is more sensitive than cytology for the detection

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of CIN2+, a degree of decreased sensitivity of urinary HPV detection could be offset by improved uptake of cervical screening (10).

The aims of this exploratory study were fourfold: to optimize urine processing for HR-HPV detection; to establish the concordance of HR-HPV detection in matched urine, vaginal and cervical samples in a colposcopy population; to compare the sensitivity of HR-HPV testing for CIN2+ detection in the same samples; and to determine the acceptability by women of self-collected versus practitioner-obtained samples for cervical screening.

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METHODS

Study participants and sample collection

Urine, vaginal and cervical samples were collected from consecutive women attending the colposcopy clinic at St Mary's Hospital, Manchester University Foundation Trust (MFT) between June and September 2015. A participant information sheet was sent with the colposcopy appointment letter to women who were 25 years of age or older and attending the colposcopy clinic for management of abnormal cervical screening results or a suspicious-looking cervix. The study was approved by the North West Research Ethics Committee (15/NW/0218) and all women gave written, informed consent to take part. Women who declined participation in the study were asked to give their reasons. Pregnant women were excluded from the study. Study samples were obtained prior to colposcopic examination of the cervix, where this was required. Self-collected first stream urine samples were collected in a sterile dry pot, followed by self-collected vaginal samples obtained using the Rovers Evalyn Brush (Netherlands). Women were asked to place the brush in the vagina, rotate it five times for sample collection and replace the brush in its casing. Practitioner-obtained cervical samples were then taken with a cervical broom immediately prior to clinically-indicated procedures. Following colposcopy, women were asked to complete a questionnaire about the acceptability of the tests performed (**Supplemental Appendix 1**).

Clinical procedures

Women were managed clinically according to NHS Cervical Screening Programme guidelines. All colposcopists were accredited for independent practice by the British Society of Colposcopy and Cervical Pathology (BSCCP). Abnormal colposcopy indicated directed punch biopsy and histological review by consultant gynecological histopathologists, who classified biopsies as normal, low grade CIN (CIN1), or high grade CIN (CIN2/3).

HPV DNA testing

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Blinded samples were delivered to the MFT Virology laboratory within four hours of collection. Cervical samples were collected using SurePath Liquid Based Cytology (LBC) and vaginal cells were suspended in solution by agitating the Evalyn brush in SurePath preservative on receipt in the laboratory. Sample aliquots were added to Abbott and Roche secondary tubes as per the manufacturers' instructions. Batches of samples were tested for high risk HPV using the Abbott RealTi*m*e (ART) and Roche Cobas 4800 (RC) assays within 48 hours and one week of sample processing, respectively. Both assays used fully automated systems that amplify and detect 14 HR-HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) simultaneously. Separate probes enable the specific detection of HPV types 16 and 18. Both assays also measure human beta globin as a control for sample cellularity, valid sample extraction and amplification. The cycle threshold (ct) is a measure of DNA concentration, with ct values inversely proportional to the amount of target nucleic acid in the sample. All samples were tested according to the manufacturers' instructions. Both assays (ART and RC) were applied to all samples, where sufficient material was available.

Optimization of urine sample processing for HR-HPV testing

Urine sample processing was optimized by comparing HR-HPV detection in samples refrigerated at 4 degrees both without preservative ('neat') and following transfer to Abbott and Roche preservative tubes ('preservative-fixed'), by dividing samples in half upon receipt by the virology laboratory for the first 50 study participants. Preservative-fixed samples were prepared using the Abbott multi-collect specimen collection kit and the Roche Cobas PCR urine specimen collection kit, according to manufacturers' instructions. The stability of neat and preservative-fixed urine samples was compared by HR-HPV testing using both assays within 48 hours, one week and one month of receipt. The ct at which HR-HPV and the internal beta globin control was detected was compared in the same samples tested after different time intervals, to determine the stability of DNA in neat and preservative-fixed urine samples. Although the receipt of neat urine samples and their rapid fixation

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and/or refrigeration within 4 hours of collection is not compatible with urine self-collection at home and postal delivery to the laboratory, this study sought to explore the potential for urinary HR-HPV detection under optimal processing and transportation conditions, to determine proof-of-principle that further studies are warranted.

Statistical analysis

HPV detection rates were calculated for each assay and sample type. Concordance between the different samples for HR-HPV positivity was determined using Cohen's kappa statistic. Agreement was slight (k<0.20), weak (k=0.21-0.40), moderate (k=0.41-0.60), substantial (k=0.61-0.80), near perfect (k=0.81-0.99), or perfect (k=1.00). The sensitivity of HR-HPV positivity for CIN2+ detection was compared between urine, vaginal and cervical samples. Sensitivity was calculated as the number of true positives divided by the sum of the number of true positives plus false negatives.

Patient and public involvement

Patients and the public were not involved in designing or conducting this research. We will work with patient support groups and forums, including the Eve Appeal and Jo's Cervical Cancer Trust, to help disseminate the results of this study.

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RESULTS

Study participants

Of 127 eligible women, 104 took part in the study (**Figure 1**). Their median age range and distribution reflected that of routine colposcopic practice, with 40% between 25 and 29 years of age. Non-participants had similar demographics to those who did agree to the study. The reasons for non-participation are shown in **Figure 1**. Triple matched samples were not available for 17 women (no vaginal sample n=8; no cervical sample n=5; no urine sample n=1; only urine sample n=2; only cervical sample n=1).

Optimization of urine sample processing for HR-HPV testing

A comparison was made between neat and preservative-fixed urine for HR-HPV detection using both ART and RC assays for the first 50 study participants. There were 44 matched neat and preservative-fixed urine sample pairs available for the ART assay. Two failed the internal beta globin control and were excluded from further analysis. A cycle threshold (ct) cut off of ≤32 was used to define HR-HPV positivity. **Table 1** demonstrates good concordance between neat and preservative-fixed urine samples (37/42, 88%, k=0.76, 95%CI 0.56-0.96).

TABLE 1: Comparison of neat versus preservative-fixed urine using the Abbott RealTi*me* (ART) assay and the Roche Cobas 4800 (RC) assay for HR-HPV detection

ART assay	**Neat positive**	**Neat negative**	**Total**
Preservative positive	19	3	22
Preservative negative	2	18	20
Total	21	21	42
RC assay	**Neat positive**	**Neat negative**	**Total**
Preservative positive	14	2	16
Preservative negative	0	6	6

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Total	14	8	22

Repeat testing of the 47 preservative-fixed urine samples approximately one month later (mean 33 days) showed no reduction in stability of HR-HPV DNA, with almost identical ct values, as shown in **Supplemental Figure 1** (mean ct 25.2 for samples tested within 48 hours versus 25.1 for samples tested at one month). This was true when all HR-HPV types, HPV 16/18 and HR-HPV other were considered separately (data not shown).

There were 40 matched neat and preservative-fixed urine sample pairs available for the RC assay. Eighteen of the neat but none of the preservative-fixed urine samples, which had been stored for up to one week prior to testing, failed the internal beta globin control and were excluded from further analysis. Thus there were 22 matched urine sample pairs available for testing with the RC assay. A cycle threshold of ≤40 was used to define HR-HPV positivity. **Table 1** shows good concordance between neat and preservative-fixed urine samples (20/22, 90%, k=0.79, 95%CI 0.52-1.0). Based on these data and the apparent instability of neat urine samples over time, all subsequent urine samples were fixed with preservative upon receipt in the lab, and neat urine samples were not considered further.

HR-HPV detection in urine, vaginal and cervical samples

Abbott RealTi*m*e assay

From the 104 women, 79 had valid HR-HPV results from matched urine, vaginal and cervical samples for the ART assay. Triple samples were not available for 17 women and invalid results were obtained for one or more sample for a further 8 women (seven vaginal samples and one cervical sample). There were no invalid results for urine samples. Directed cervical biopsies were taken from 66/79

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(84%) of these women because of abnormal cytology. Histological analysis classified the biopsies within normal limits (31/66, 47%), CIN1 (17/66, 26%), CIN2 (10/66, 15%) and CIN3 (8/66, 12%).

HR-HPV was detected in 48/79 (61%), 54/79 (68%) and 56/79 (71%) of urine, vaginal and cervical samples respectively. HPV 16/18 was detected in 21/79 (27%), 24/79 (30%) and 23/79 (29%). Concordance between the three sample types was found in 62/79 (79%), as shown in **Table 2**. According to the manufacturer's cut-off of 32ct, the sensitivity for detecting CIN2+ was 16/18 (89%) for both cervical and vaginal samples and 15/18 (83%) for urine samples. Concordance between urine and cervical samples was 82% (65/79; k=0.61; 95% CI 0.43-0.79) and between vaginal and cervical samples was 90% (71/79; k=0.76; 95% CI 0.61-0.92). Only one CIN2+ lesion was 'missed' from the 8 women with invalid results by the ART assay (a vaginal sample).

TABLE 2: HR-HPV detection in preservative-fixed urine, vaginal and cervical samples by the Abbott RealTi*m*e (ART) assay and the Roche Cobas 4800 (RC) assay

		Cervical positive		Cervical negative	
		Vaginal positive	**Vaginal negative**	**Vaginal positive**	**Vaginal negative**
ART	**Urine positive**	43[a]	2	2	1
	Urine negative	8[b]	3	1	19[c]
RC					
	Urine positive	44[d]	0	1	0
	Urine negative	4	0	1	16[e]

[a]9 CIN2, 6 CIN3; [b]1 CIN3; [c]1 CIN2, 1 CIN3; [d]8 CIN2, 7 CIN3; [e]1 CIN2, 1 CIN3

A comparison of the ct values for detection of the internal beta globin control demonstrated lower DNA concentrations in urine compared with vaginal and cervical samples (**Figure 2a**). The mean ct value for urine, vaginal and cervical samples was 25.6, 20.9 and 23.1 respectively, with similar results

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for samples which showed positive results for HPV 16/18 or HPV other (25.3, 20.6 and 20.9 respectively) (**Figure 2b**).

Roche Cobas 4800 assay

From the 104 women, 66 had valid HR-HPV results from matched urine, vaginal and cervical samples for the RC assay. Triple samples were not available for 17 women, there was insufficient volume for the RC assay for 8 cervical and 3 urine samples, and invalid results were obtained for one or more sample for 10 women (two vaginal and ten cervical samples). There were no invalid results for urine samples. Cervical biopsies were taken for 54/66 (82%) of these women and histological analysis classified these as normal (24/54, 44%), CIN1 (14/54, 26%), CIN2 (8/54, 15%) and CIN3 (8/54, 15%).

HR-HPV was detected in 45/66 (68%), 50/66 (76%) and 48/66 (73%) of urine, vaginal and cervical samples respectively. HPV 16/18 was detected in 16/66 (24%), 21/66 (32%) and 19/66 (29%). Concordance between the three sample types was found in 60/66 (91%), as shown in **Table 2**. According to the manufacturer's cut-off of 40ct, the sensitivity for detecting CIN2+ was 15/17 (88%) for all three sample types. Concordance between urine and cervical samples was 92% (61/66, k=0.82, 95% CI 0.67–0.97) and between vaginal and cervical samples was 97% (64/66, k=0.77, 95% CI 0.60-0.92). No CIN2+ lesions were 'missed' amongst women with invalid results by the RC assay.

A comparison of the mean ct values for detection of the internal beta globin control again demonstrated lower DNA concentrations in urine (**Figure 2c**). The mean ct values for urine, vaginal and cervical samples were 31.7, 27.5 and 29.9 respectively, with similar findings with concordant positive results for HPV 16/18 or HPV other (33.6, 28.1 and 27.7 respectively) (**Figure 2d**).

Acceptability of urine, vaginal and cervical sampling for cervical screening

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A total of 98 women answered the acceptability questionnaire (**Supplemental Appendix 1**). Most of the participants had heard about HPV before (yes, 73; no, 17; don't know, 1; not answered, 7). Participants were confident about providing self-obtained vaginal and urine samples for HPV testing and were not excessively concerned about the accuracy of the test in these samples (**Figure 3**). In general, women were more confident about providing a urine sample than a vaginal sample for HPV testing. When asked which sample they would prefer to provide, more women chose urine (39) than either vaginal (32) or cervical samples (17; no preference, 1; not answered, 9).

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DISCUSSION

This study found good concordance for HR-HPV detection between preservative-fixed urine, vaginal and cervical samples amongst women attending a colposcopy clinic. Urine showed similar, albeit slightly inferior, sensitivity to matched vaginal and cervical samples for CIN2+ detection using both ART and RC assays. Cervical screening by urinary and vaginal HR-HPV detection was considered acceptable by women.

This was an exploratory study of women attending the colposcopy clinic for the management of abnormal cervical screening results or a suspicious-looking cervix. Forty percent of the participants were under 30 years of age. The prevalence of HR-HPV was therefore sufficiently high to allow comparison between the three sampling methods. Matched urine, vaginal and cervical samples taken at the same visit and immediately prior to colposcopic assessment, eliminated the possibility that HPV had been acquired or cleared between tests. The order of obtaining matched samples meant that cross-contamination was unlikely. We found that preservative-fixed urine was suitable for HR-HPV detection by both ART and RC assays within 48 hours and up to one month following its receipt. The consistent results showed that preservative-fixed urine can be handled in the same way as cervical samples without compromising analytical sensitivity.

The relatively small size of the study means that urine testing needs to be fully validated in larger cohorts. The colposcopy clinic setting facilitated an assessment of the analytical sensitivity of urinary HR-HPV testing but the true value of urine-based cervical screening is likely to be its ability to improve cervical screening uptake by non-attenders. The prevalence of HR-HPV is much lower in the general population, which could be expected to affect the sensitivity and negative predictive value of the test. Studies designed to evaluate the accuracy and clinical utility of urinary HR-HPV detection in the primary cervical screening population are therefore required (11). Urinary self-sampling was

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acceptable to women attending colposcopy clinic; those who declined participation did so for other reasons. An important limitation of this study is that self-sampling took place before colposcopy, which may have affected women's confidence in the accuracy of self-sampling. The acceptability of urinary self-sampling to cervical screening non-attenders remains unclear, as does the ability of urinary HR-HPV detection to improve uptake. Defining its acceptability in 'hard to reach' groups is challenging but crucial to its further development as an alternative test for cervical screening. Forty percent of our participants were under 30 years of age, a group of women that stands to benefit most from strategies to improve uptake, given their declining participation in cervical screening programmes (5).

Previous studies have looked at urine sampling techniques (12, 13) but few have reported the optimization of urine processing and storage for HR-HPV detection. Here we show that preservative-fixed urine has improved stability and can be stored prior to testing for much longer than neat urine, which rapidly deteriorates after 48 hours. This may explain the improved clinical performance of urinary HR-HPV reported here compared to studies that tested neat urine (8). In total, 45% of neat urine samples tested on the RC assay at one week were invalid compared to only 2% of samples tested within 48 hours of collection by ART assay. This can be explained by an increase in PCR inhibitors over time. Preservative-fixed urine stored at 4^{o}C showed only one invalid result using the ART assay amongst 104 urine samples tested within 48 hours, and there were no invalid results using the RC assay amongst 98 urine samples tested between 7 and 14 days. Repeat testing at one month showed consistent results. Whilst the requirement to preserve urine samples challenges the ideal scenario of home collection and postage of urine samples to the laboratory, it would not preclude self-collection within Primary Care, where preservative can be safely added shortly afterwards.

The analytical and clinical sensitivity of the RC and ART assays for cervical HR-HPV detection are well documented (14-16), and both assays are approved for primary HPV screening, HPV triage of minor

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cytological abnormalities and test of cure in the NHS Cervical Screening Programme. Neither assay is currently CE marked for urinary testing and whilst the data in this pilot study are consistent with published studies, more work is needed to define viral load thresholds of clinical utility for the detection of CIN2+ (17, 18). Raising the threshold of the ART assay above 32ct (manufacturer's cut-off) did pick up four more positive urine samples but did not identify the single undetected CIN2+, which suggests that the manufacturer's cut-offs are appropriate for urine samples. The analytical sensitivity for HR-HPV in urine, vaginal and cervical samples were similar though a lower proportion of HR-HPV was detected in urine samples compared with vaginal samples.

Barriers to cervical screening include embarrassment, fear, inconvenience and the view held by some women that they are not at risk (4). Offering an alternative means of cervical screening can encourage non-attenders to be screened, either by 'nudging' them to undergo cervical sampling or by facilitating self-sampling at home (5). Urine-based testing is already widely used for the detection of chlamydia and is generally trusted and acceptable to women. The disadvantage of self-testing is that reflex cytology is not possible in the event of a positive HR-HPV test, however, data suggest that women who screen positive for HR-HPV are highly likely to attend for cervical cytology (19). Urine-based testing may also be cheaper than standard cervical screening tests because it does not require an appointment, a practitioner or any special equipment and it lends itself to high throughput testing. Thus it could also be suitable in resource poor settings where the infrastructure required to underpin screening by cervical cytology is lacking.

CONCLUSIONS

Urinary HR-HPV testing has good sensitivity for the detection of CIN2+ in a colposcopy clinic cohort. Our study suggests that vaginal HR-HPV testing is slightly more sensitive than urine HR-HPV testing, however urine self-sampling may be more acceptable to cervical screening non-attenders than vaginal self-sampling. Future studies are warranted to assess the performance and acceptability of

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urine self-sampling in comparison with vaginal self-sampling in the general screening population.

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LIST OF ABBREVIATIONS

ART	Abbott RealTi*m*e
BSCCP	British Society of Colposcopy and Cervical Pathology
CIN	cervical intraepithelial neoplasia
ct	cycle threshold
HR	high risk
HPV	human papillomavirus
LBC	liquid based cytology
PCR	polymerase chain reaction
RC	Roche Cobas 4800
UK NSC	UK National Screening Committee

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DECLARATIONS

Ethics approval and consent to participate

The study was approved by the North West Research Ethics Committee (15/NW/0218) and all women gave written, informed consent to take part.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

HK is Chair of the Advisory Committee for Cervical Screening (PHE), but the views expressed in this manuscript are those of the author and not Public Health England.

Funding

EC is a National Institute for Health Research (NIHR) Clinician Scientist (NIHR-CS-012-009). This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Author contributions

AS performed the laboratory analyses, data interpretation and wrote the first draft of the manuscript. SF and KB recruited and sampled the study participants and contributed to data interpretation. HK and EC designed the study and contributed to data interpretation and manuscript preparation. All authors reviewed the final version of the manuscript.

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Acknowledgements

The authors would like to thank all the women who participated in this study and donated their samples and data for research. We would like to thank Mrs. Linsey Nelson for her support with obtaining all the necessary regulatory approvals and for data management.

Data sharing statement

Data are available and will be shared upon reasonable request.

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FIGURE 1: Demographics of study participants and reasons for declining participation. Key: PG, postgraduate; UG, undergraduate

FIGURE 2: A comparison of the mean ct values for detection of the internal beta globin control and HR-HPV in urine, vaginal and cervical samples using the ART (a, b) and RC assays (c, d).

FIGURE 3: The acceptability of urine and vaginal self-obtained samples for cervical screening

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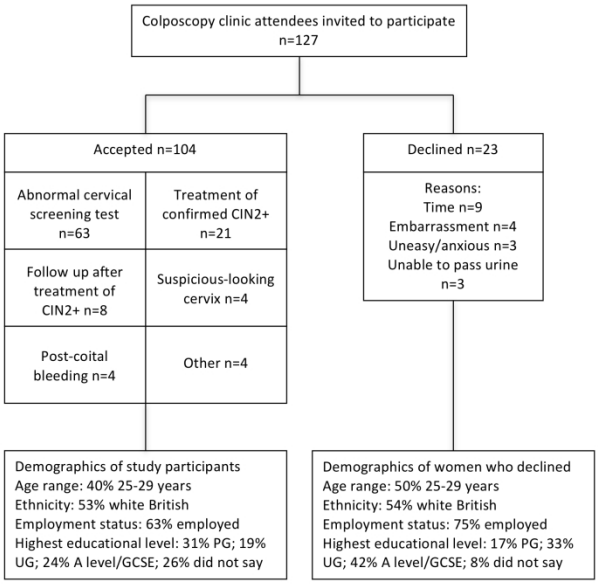

FIGURE 1: Demographics of study participants and reasons for declining participation. Key: PG, postgraduate; UG, undergraduate

209x296mm (300 x 300 DPI)

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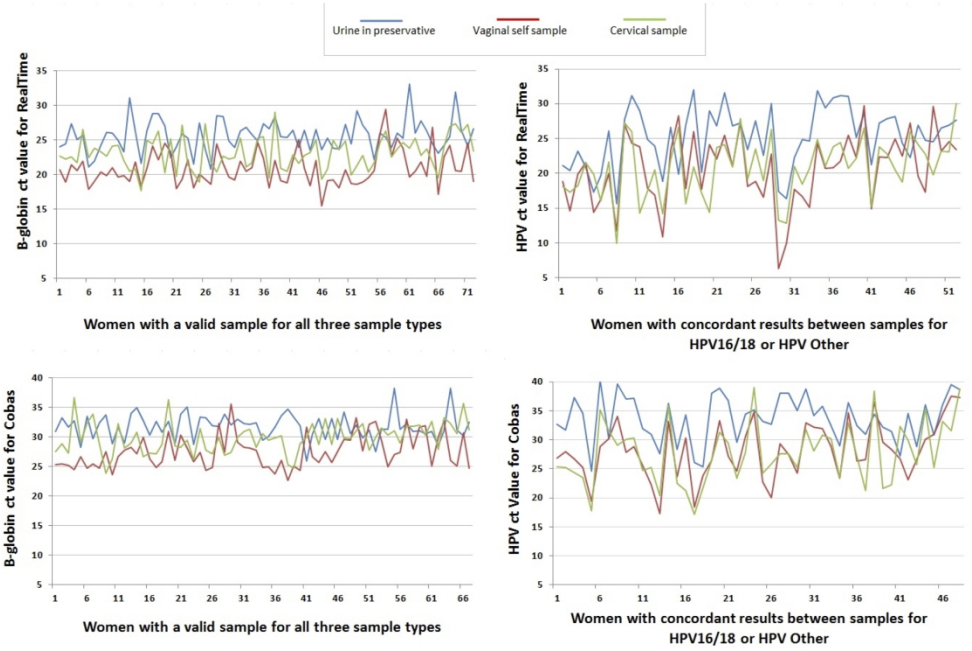

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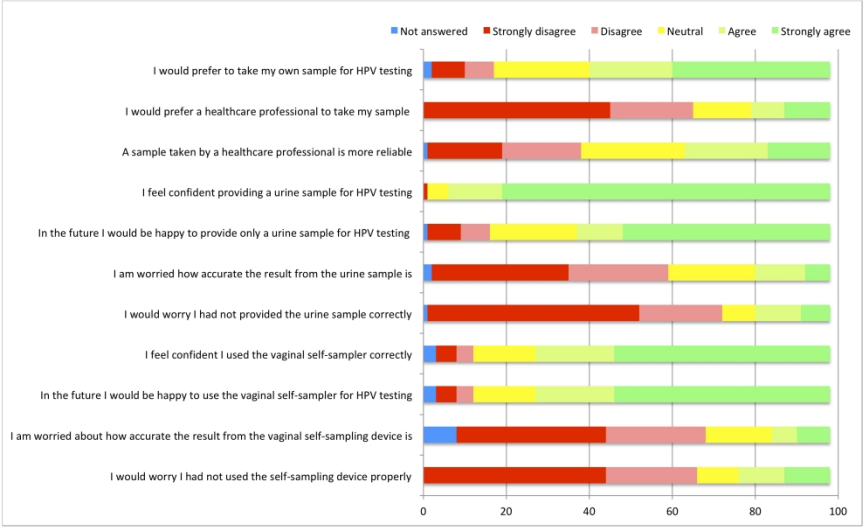

FIGURE 3: The acceptability of urine and vaginal self-obtained samples for cervical screening

297x209mm (300 x 300 DPI)

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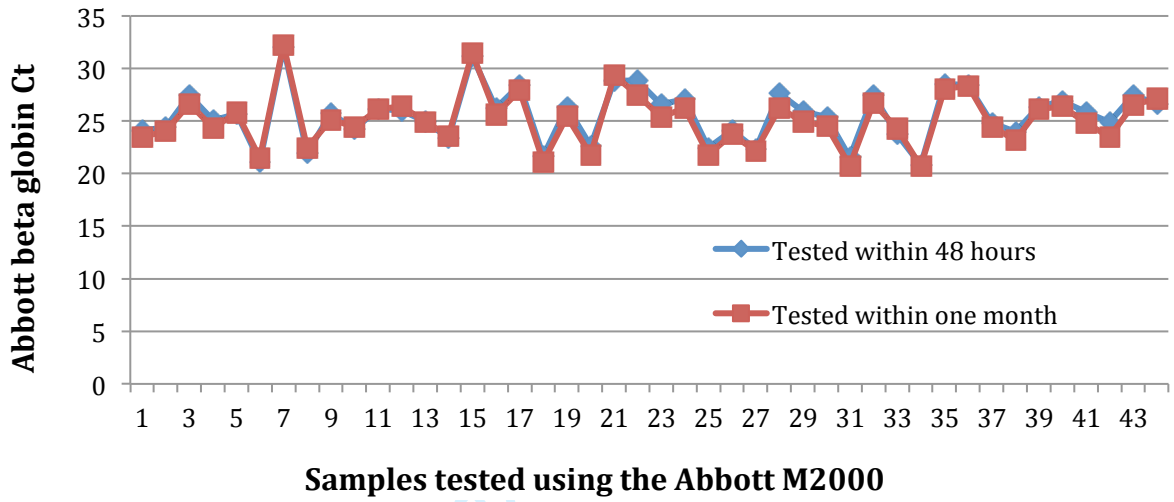
SUPPLEMENTAL FIGURE 1: Detection of internal beta globin control in preservative-fixed urine using Abbott RealTi*me* assay within 48 hours and after one month showed no reduction in DNA stability over time



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MANCHESTER 1824 Number:…… Central Manchester University Hospitals NHS NHS Foundation Trust

Urine Testing for HPV: the UVC pilot study – Questionnaire

Version One, DATE

Before completing this questionnaire please make sure you have read the information sheet. By completing this questionnaire you consent to take part in the study. To complete the questionnaire please circle the answer most applicable to you in each question, tick the correct box or write in the space provided.

Previous Knowledge of HPV

Before today had you previously heard of HPV (Human Papillomavirus)? Yes No Unsure

About the tests today

Would you have liked more information about how to provide the urine sample? Yes No

Would you have liked more information about how to use the self-sampling device? Yes No

How much do you agree with these statements? Please circle the appropriate number for each statement.

	Strongly Disagree				***Strongly Agree***
I feel confident providing a urine sample to be tested for HPV.	1	2	3	4	5
I would worry I had not provided the urine sample correctly.	1	2	3	4	5
I am worried how accurate the result from the urine sample is.	1	*2*	*3*	*4*	*5*
I feel confident I used the self-sampling device correctly.	1	2	3	4	5
I would worry I had not used the self-sampling device properly.	1	2	3	4	5
I am worried about how accurate the result from the self-sampling device is.	1	2	3	4	5
I would prefer a health professional to take my sample than to take a sample myself.	1	2	3	4	5
A sample taken by a health professional is more reliable.	1	2	3	4	5
I would prefer to take my own sample.					
In the future I would be happy to use only a urine sample to be tested for HPV.	1	2	3	4	5
In the future I would be happy to be tested for HPV from a sample taken by myself using the vaginal HPV self-sampler.	1	2	3	4	5
I felt I understood the instructions that were given to me.	1	2	3	4	5
I was happy to provide three samples.	1	2	3	4	5

Which test would you prefer to take?

Urine test Using self –sampler Clinician taken sample

What would be your second choice?

Urine test Using self –sampler Clinician taken sample

Which of these words describes how you felt about providing a urine sample? (Please circle all that apply)

Uncomfortable It was easy Embarrassed Private Reliable Convenient

Which of these words describes how you felt about using the self-sampling test? (Please circle all that apply)

Uncomfortable It was easy Embarrassed Private Reliable Convenient

Which of these words describes how you felt about having a health professional take a sample? (Please circle all that apply)

Uncomfortable It was easy Embarrassed Private Reliable Convenient

About you

What age group do you belong to? (shown in years)

24 and under 25-29 30-34 35-39 40-44 45-49 50-54 55-59 60-64 65-69 70+

Which of the following best describes your ethnicity? Please circle your answer below

White British	Indian	Caribbean	White and Black Caribbean
White Irish	Pakistani	African	White and Black African
Any other White background	Bangladeshi	Any other Black background	White and Asian
Chinese	Any other Asian background		Any other mixed background

Any Other Ethnic Group (please provide details) …….. Prefer not to say

How would you best describe your employment status?

Employed Unemployed Student Full time parent Retired

Which of these qualifications do you have? Please circle all that apply

GCE O-level/GCSE A-level or equivalent Undergraduate Postgraduate

Other (please specify) …….

Thank you for taking the time to complete this questionnaire. Please place it in the white envelope given to you and put it in the box marked 'Questionnaires' or hand it in the envelope to the clinic receptionist.

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	2
ABSTRACT			
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	2
INTRODUCTION			
	3	Scientific and clinical background, including the intended use and clinical role of the index test	5
	4	Study objectives and hypotheses	6
METHODS			
Study design	**5**	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	7
Participants	**6**	Eligibility criteria	7
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	7
	8	Where and when potentially eligible participants were identified (setting, location and dates)	7
	9	Whether participants formed a consecutive, random or convenience series	7
Test methods	**10a**	Index test, in sufficient detail to allow replication	7
	10b	Reference standard, in sufficient detail to allow replication	7
	11	Rationale for choosing the reference standard (if alternatives exist)	7
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	8
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	8
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	8
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	7
Analysis	**14**	Methods for estimating or comparing measures of diagnostic accuracy	8
	15	How indeterminate index test or reference standard results were handled	
	16	How missing data on the index test and reference standard were handled	
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	
	18	Intended sample size and how it was determined	
RESULTS			
Participants	**19**	Flow of participants, using a diagram	Figure 1
	20	Baseline demographic and clinical characteristics of participants	Figure 1
	21a	Distribution of severity of disease in those with the target condition	11, 13
	21b	Distribution of alternative diagnoses in those without the target condition	Figure 1
	22	Time interval and any clinical interventions between index test and reference standard	7
Test results	**23**	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	12
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	10-13
	25	Any adverse events from performing the index test or the reference standard	
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	15, 16
	27	Implications for practice, including the intended use and clinical role of the index test	15, 16, 17
OTHER INFORMATION			
	28	Registration number and name of registry	7
	29	Where the full study protocol can be accessed	
	30	Sources of funding and other support; role of funders	19

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STARD 2015

AIM

STARD stands for "Standards for Reporting Diagnostic accuracy studies". This list of items was developed to contribute to the completeness and transparency of reporting of diagnostic accuracy studies. Authors can use the list to write informative study reports. Editors and peer-reviewers can use it to evaluate whether the information has been included in manuscripts submitted for publication.

EXPLANATION

A **diagnostic accuracy study** evaluates the ability of one or more medical tests to correctly classify study participants as having a **target condition.** This can be a disease, a disease stage, response or benefit from therapy, or an event or condition in the future. A medical test can be an imaging procedure, a laboratory test, elements from history and physical examination, a combination of these, or any other method for collecting information about the current health status of a patient.

The test whose accuracy is evaluated is called **index test.** A study can evaluate the accuracy of one or more index tests. Evaluating the ability of a medical test to correctly classify patients is typically done by comparing the distribution of the index test results with those of the **reference standard**. The reference standard is the best available method for establishing the presence or absence of the target condition. An accuracy study can rely on one or more reference standards.

If test results are categorized as either positive or negative, the cross tabulation of the index test results against those of the reference standard can be used to estimate the **sensitivity** of the index test (the proportion of participants *with* the target condition who have a positive index test), and its **specificity** (the proportion *without* the target condition who have a negative index test). From this cross tabulation (sometimes referred to as the contingency or "2x2" table), several other accuracy statistics can be estimated, such as the positive and negative **predictive values** of the test. Confidence intervals around estimates of accuracy can then be calculated to quantify the statistical **precision** of the measurements.

If the index test results can take more than two values, categorization of test results as positive or negative requires a **test positivity cut-off**. When multiple such cut-offs can be defined, authors can report a receiver operating characteristic (ROC) curve which graphically represents the combination of sensitivity and specificity for each possible test positivity cut-off. The **area under the ROC curve** informs in a single numerical value about the overall diagnostic accuracy of the index test.

The **intended use** of a medical test can be diagnosis, screening, staging, monitoring, surveillance, prediction or prognosis. The **clinical role** of a test explains its position relative to existing tests in the clinical pathway. A replacement test, for example, replaces an existing test. A triage test is used before an existing test; an add-on test is used after an existing test.

Besides diagnostic accuracy, several other outcomes and statistics may be relevant in the evaluation of medical tests. Medical tests can also be used to classify patients for purposes other than diagnosis, such as staging or prognosis. The STARD list was not explicitly developed for these other outcomes, statistics, and study types, although most STARD items would still apply.

DEVELOPMENT

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on http://www.equator-network.org/reporting-guidelines/stard.

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