Comparison of urine specimen collection times and testing fractions for the detection of high-risk human papillomavirus and high-grade cervical precancer

V. Senkomago [a], A.C. Des Marais [a], L. Rahangdale [b,d], C.R.T. Vibat [c], M.G. Erlander [c], J.S. Smith [a,d,*]

[a] Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, NC, USA
[b] Department of Obstetrics and Gynecology, University of North Carolina at Chapel Hill, NC, USA
[c] Trovagene Inc., San Diego, CA, USA
[d] Lineberger Comprehensive Cancer Center, Chapel Hill, NC, USA

ARTICLE INFO

Article history:
Received 27 June 2015
Received in revised form 27 October 2015
Accepted 1 November 2015

Keywords:
Human papillomavirus
Urine
Cervical cancer screening

ABSTRACT

Background: Urine testing for high-risk human papillomavirus (HR-HPV) detection could provide a non-invasive, simple method for cervical cancer screening.
Objectives: We examined whether HR-HPV detection is affected by urine collection time, portion of urine stream, or urine fraction tested, and assessed the performance of HR-HPV testing in urine for detection of cervical intraepithelial neoplasia grade II or worse (CIN2+).
Study design: A total of 37 female colposcopy clinic attendees, ≥30 years, provided three urine samples: "first void" urine collected at home, and "initial stream" and "mid-stream" urine samples collected at the clinic later in the day. Self- and physician-collected brush specimens were obtained at the same clinic visit. Colposcopy was performed and directed biopsies obtained if clinically indicated. For each urine sample, HR-HPV DNA testing was conducted for unfractionated, pellet, and supernatant fractions using the Trovagene test. HR-HPV mRNA testing was performed on brush specimens using the Aptima HPV assay.
Results: HR-HPV prevalence was similar in unfractionated and pellet fractions of all urine samples. For supernatant urine fractions, HR-HPV prevalence appeared lower in mid-stream urine (56.8%[40.8–72.7%]) than in initial stream urine (75.7%[61.9–89.5%]). Sensitivity of CIN2+ detection was identical for initial stream urine and physician-collected cervical specimen (89.9%[95%CI = 62.7–99.6%]), and similar to self-collected vaginal specimen (79.1%[48.1–96.6%]).
Conclusion: This is among the first studies to compare methodologies for collection and processing of urine for HR-HPV detection. HR-HPV prevalence was similar in first void and initial stream urine, and was highly sensitive for CIN2+ detection. Additional research in a larger and general screening population is needed.

1. Background

Progression to invasive cervical cancer (ICC) is highly preventable with sufficient screening and treatment [1]. However, screening coverage remains low in low and middle-income countries, and a notable proportion of women in high-income countries are not screened according to current guidelines [2]. In the United States (US), an estimated 56% of incident ICC is due to insufficient screening [3]. In 2012, 11.4% of US women age 21–65 years reported no history of screening within the preceding five years [4].

Current cervical cancer screening strategies in the US include cytology (Pap testing) or co-testing—cytology plus testing for high-risk human papillomavirus (HR-HPV)–which both require pelvic examination by trained medical personnel. Self-collection of specimens for HR-HPV testing can be performed outside a health facility to increase ease of and access to screening uptake [5], and has been found highly acceptable in different populations [6]. Urine collection for HR-HPV detection could provide an especially simple, non-invasive method for screening women reluctant to undergo a

* Corresponding author at: Department of Epidemiology, 103 McGavren Greenberg CB7435, University of North Carolina, Chapel Hill, NC, 27599−7435, USA.
E-mail address: JenniferS@unc.edu (J.S. Smith).

http://dx.doi.org/10.1016/j.jcv.2015.11.005

pelvic examination. HR-HPV detection in urine samples and cervical scrapes has been found to be similar [7,8], however, HR-HPV prevalence in urine has ranged considerably among studies, likely due to lack of standardization of urine collection and handling, and different HR-HPV extraction and amplification techniques [9].

Few data are available which examine differences in HR-HPV detection in urine, stratified by time and method of collection, and only one study examined HR-HPV detection in supernatant versus pellet fractions [10]. Understanding variations in HR-HPV detection in urine by sample collection method or fractions tested is essential for developing urine collection and processing procedures for future screening implementation.

2. Objectives

The HR-HPV detection test (HPV HR) from Trovagene (San Diego, CA) uses a preservative buffer and a novel detection assay that targets the HPV E1 region to amplify and detect short fragments of HPV DNA in urine [11]. We present here data from a pilot study to examine HR-HPV detection in urine collected at different times (first urination of the day versus initial stream and mid-stream collected later the same day) and in different urine fractions (supernatant, pellet, and unfractionated) using the Trovagene HPV HR test. We also examine the validity of HPV testing in the different urine samples for the detection of histologically-confirmed CIN2+.

3. Study design

3.1. Study population

This pilot study was conducted among 37 non-pregnant women, ≥30 years, who attended the colposcopy clinic at the UNC Women's Hospital between October 2013 and May 2014 for follow-up of results of abnormal cytology or persistent HPV infection or treatment by loop electrical excision procedure (LEEP). Potentially eligible women were identified by chart review and contacted via phone in advance of their clinic appointment to be invited to participate.

3.2. Specimen collection

Prior to their appointment, participants were sent a urine collection kit consisting of a collection cup, preservative solution, illustrated collection instructions, and forms to complete for informed consent and HIPAA authorization. Women were instructed to collect approximately 60 ml of urine from the beginning of the stream of their first urination ("first void") on the morning of their clinic appointment, add preservative (8 ml of EDTA), and bring the urine sample to their appointment. At their appointment, participants were instructed to provide two additional urine samples: 20 ml collected at the beginning of the urine stream ("initial stream"), and 60 ml collected from the middle of the same stream ("mid-stream"). Study staff added the preservative (8 ml of EDTA) immediately to urine samples following collection [11,12].

Participants then self-collected a cervicovaginal sample by inserting a Viba brush (Rovers Medical Devices BV, The Netherlands) into the upper vagina, rotating and removing it, and placing the brush head directly into Aptima sample transport medium (Hologic Inc., Bedford, MA). Following self-collection, participants completed a questionnaire collecting demographic and acceptability measures. Participants then underwent pelvic exam, during which the physician collected a cervical sample using the Viba brush and preserved it in Aptima medium for HR-HPV testing, and then a colposcopic examination was performed. Cervical disease status of the women was based on histological analysis of the tissue. Directed biopsies were performed in women with visible lesions and treatment by LEEP was performed, as indicated. Women without visible lesions were categorized as disease negative (<CIN2) for data analyses.

Urine samples were shipped overnight to Trovagene for HR-HPV DNA testing. Physician- and self-collected specimens were shipped to Hologic for HR-HPV mRNA testing. Cervical biopsies and tissues removed during LEEP underwent histological assessment at UNC and were classified using standard pathology grading. Women were referred to follow-up screening or treatment per standard clinic procedures. This study was approved by the Institutional Review Board at UNC-Chapel Hill.

3.3. Laboratory analyses

For each woman, a total of nine HR-HPV urine test results were obtained: three urine fractions (unfractionated, pellet, and supernatant fractions) were tested from each of the three urine samples (first void, initial stream, and mid-stream). Urine samples were shaken and 0.5 ml removed as the "unfractionated" aliquot. Up to 40 ml of the sample was then centrifuged to obtain "pellet" and "supernatant" fractions. The pellets were resuspended in 0.5 ml of supernatant. DNA was extracted from 0.5 ml of each fraction using the QIAamp MinElute Virus Vacuum Kit (QIAGEN, Germantown, MD) per the manufacturer's instructions. Isolated DNA (5 ul) was tested with the HPV HR test (Trovagene Inc., San Diego, CA), which amplifies a conserved region in the E1 gene of 13HR-HPV genotypes (16,18,31,33,35,39,45,51,52,56,58,59 and 68), as well as RNaseP (control). Amplicons were subjected to capillary electrophoresis for fragment size analysis on the ABI 3130 instrument (ThermoFisher, Carlsbad, CA). The limit of detection of the Trovagene assay is 500 copies of high-risk HPV DNA.

Physician- and self-collected specimens were tested for HR-HPV mRNA using the Aptima HPV assay, which qualitatively detects E6/E7 mRNA of 14HR-HPV types (16,18,31,33,35,39,45,51, 52,56,58,59,66 and 68) [13].

3.4. Statistical analyses

HR-HPV prevalence estimates, with 95% confidence intervals (CIs), were calculated for each urine sample type and fraction. We conducted pairwise comparisons with McNemar's test to assess differences in HR-HPV prevalence between urine sample types (by the same fraction) and between urine fractions (within same sample). Cohen–Kappa values were calculated to assess agreement between urine samples. Median unbiased estimates and associated mid-P 95% CIs were computed for sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for CIN2+ detection, stratified by sample type [14]. Given the small sample size of this study, median-unbiased estimates were chosen to provide better approximations to large-sample analyses than maximum likelihood estimates. Generalized Estimating Equations (GEE) accounting for repeated measures and chi-square test of equal proportions were used to assess differences in participants' preferences of urine versus brush self-collection.

4. Results

4.1. Participant characteristics

Median participant age was 42 years (range 30–63 years); most (N = 15; 41%) were non-Hispanic White, 12(32%) were Hispanic, and 9(24%) were African-American, with one unspecified. Most women had a high school education or greater (n = 29; 78%) and were unmarried [11(30%) single; 10(27%) divorced/separated].

Table 1
High-risk (HR) human papillomavirus (HPV) results, stratified by urine collection times and urine fractions tested[a].

Type of urine sample	HR-HPV positive women, N (%)					
	First morning void at home (*N* = 37)		Initial stream sample at clinic (*N* = 37)		Mid-stream sample at clinic (*N* = 37)	
	No. HR-HPV+	(%) 95% CI	No. HR-HPV+	(%) 95% CI	No. HR-HPV+	(%) 95% CI
Urine fraction						
Unfractionated	24	64. 9 (49.5–80.2)	27	73.0 (58.7–87.3)	26	70.3 (55.5–85.0)
Pellet	25	67.6 (52.5–82.7)	29	78.4 (65.1 91.6)	27	73.0 (58.7–87.3)
Supernatant	27	73.0 (58.7–87.3)	28	75.7 (61.9–89.5)	21	56.8 (40.8–72.7)

[a] A total of nine HR-HPV test results were obtained for each participant: three different fractions (unfractionated (not centrifuged), pellet and supernatant) obtained from each of the three urine sample types (morning void, initial stream and mid-stream) collected per participant.

Nine women (24%) had private insurance, 9(24%) had medicaid/medicare, and 19(51%) were uninsured.

4.2. HR-HPV detection, stratified by urine sample type and by urine fraction

HR-HPV prevalence was similar in unfractionated portions of the three types of urine samples: 64.9%(49.5–80.2%) in first void, 73.0%(58.7–87.3%) in initial stream, and 70.3%(55.5–85.0%) in mid-stream (p-value range for pairwise comparisons: 0.26–0.80)(Table 1, Appendix A). HR-HPV detection was similar in all pellet fractions: 67.6%(52.5–82.7%) in first void, 78.4%(65.1–91.6%) in initial stream, and 73.0%(58.7–87.3%) in mid-stream (*p*-values range: 0.102–0.414). In supernatant fractions, HR-HPV prevalence was similar for first void (73.0%[58.7–87.3%]) and initial stream (75.7%[61.9–89.5%]) samples, albeit lower in mid-stream samples (56.8%[40.8–72.7%]) than in initial stream samples (*p*-value = 0.035).

HR-HPV prevalence estimates for unfractionated, pellet, and supernatant fractions were similar within the first void and initial stream samples. However, in mid-stream samples, HR-HPV prevalence in supernatant fractions (56.8%[40.8–72.7%]) was significantly lower than in pellet fractions (73.0%[58.7–87.3%])(*p*-value = 0.034), but was not different from unfractionated samples (70.3%[55.5–85.0%])(*p*-value = 0.062).

4.3. Detection of high-grade cervical lesions

The validity of HPV testing performance on urine for CIN-2+ detection was assessed using the unfractionated initial stream samples, given that HR-HPV prevalence in unfractionated urine samples was similar to other fractions and prevalence in initial stream was similar to other sample types. The sensitivity of HR-HPV DNA testing in urine for CIN2+ detection was high (89.9%[95% CI = 62.7–99.6%]), identical to that of mRNA testing of physician-collected specimens, and comparable to that of self-collected genital specimens (79.1%[48.1–96.6%]) (Fig. 1). Specificity of HR-HPV DNA in urine was relatively low (34.8%[18.4–54.1%]), but comparable to specificity of HR-HPV mRNA testing on physician-collected (42.4%[24.6–61.6%]) and self-collected genital specimens (46.2%[27.9–65.2%]). HR-HPV testing in urine, self-collected, and physician-collected specimens had low, but comparable PPVs of 37.2%(20.6–56.2%), 38.3%(19.5–59.8%) and 40.1%(22.4–59.8%), respectively. NPV estimates for all tests were high: 88.9%(59.7–99.5%) for urine, 84.9%(60.3–97.6%) for self-collected specimens and 90.7%(65.3–99.6%) for physician-collected specimens.

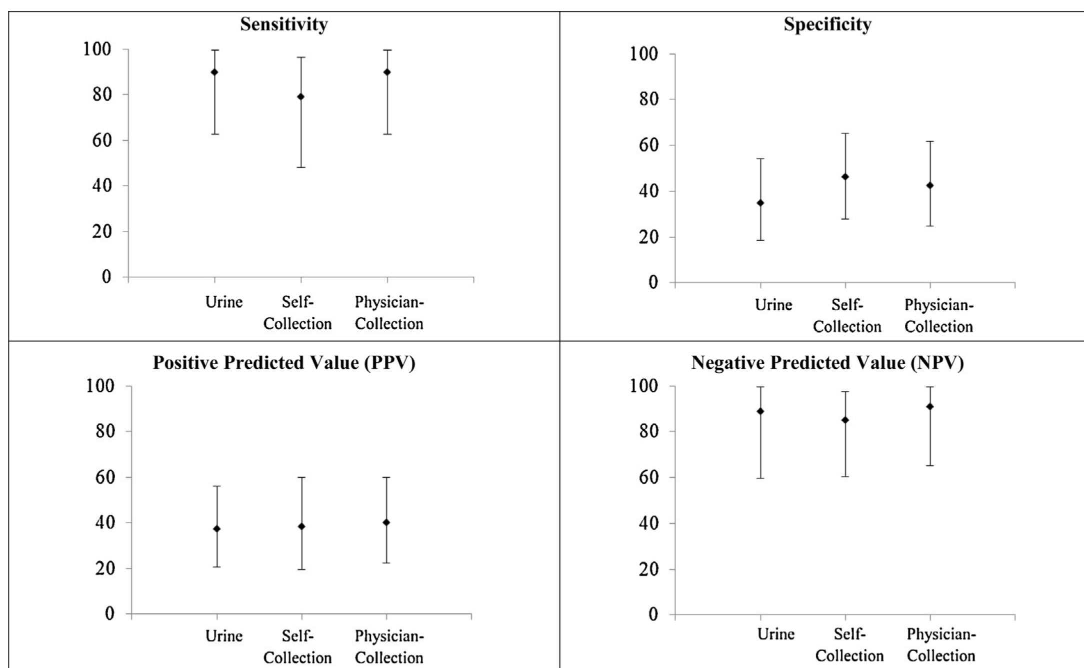

Fig. 1. Sensitivity, specificity, PPV, and NPV for CIN2+ detection by different tests among 37 women attending a colposcopy clinic PPV= Positive predicted value; NPV = Negative predicted value. Estimates are median unbiased estimates and corresponding mid-P 95% confidence intervals. Urine results shown are for initial stream unfractionated urine sample.

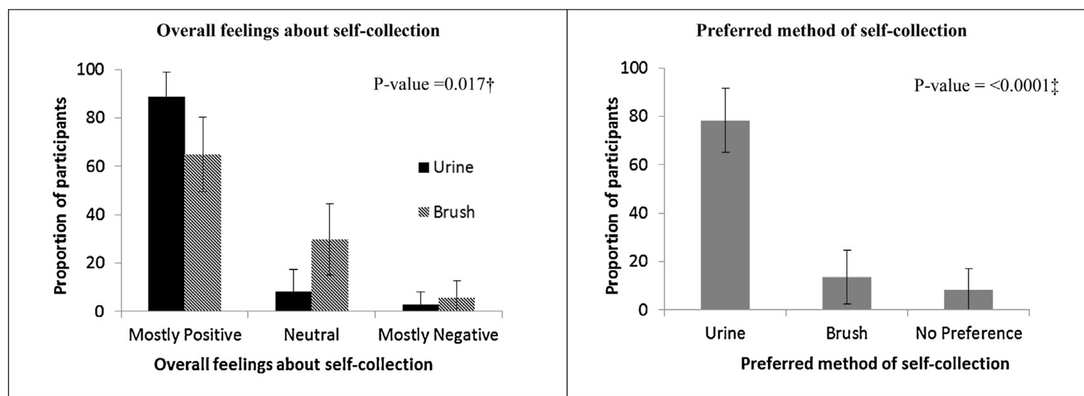

Fig. 2. Acceptability of urine versus brush self-collection for HR-HPV detection among 37 women attending a colposcopy clinic*.
* Data are proportions and corresponding 95% confidence intervals.
† *P*-value for test of equal proportions (Generalized Estimating Equations accounting for repeated measures). N for urine self-collection: mostly positive = 32; neutral = 3; mostly negative = 1. N for brush self-collection: mostly positive = 23; neutral = 11; mostly negative = 2. One woman did not report overall feelings about self-collection.
‡ *P*-value for chi-square test of equal proportions. N for urine preference = 29; brush preference = 5; no preference = 3.

4.4. Acceptability of HR-HPV testing in urine

A greater proportion of participants reported having mostly positive feelings about urine collection than brush self-collection (89% versus 65%), and more women reported neutral or negative feelings about brush self-collection than urine collection (neutral = 8% versus 30%, and mostly negative = 3% versus 5%, respectively) (GEE *p*-value = 0.017) (Fig. 2). Most women (*n* = 29, 78.4%) preferred urine collection to brush self-collection (chi-square *p*-value < 0.001) and reported being comfortable with receiving the urine collection kit in the mail (*n* = 32, 86.5%).

5. Discussion

Given that HR-HPV testing was approved by the FDA in April 2014 for primary screening of women ≥25 years, further evaluation of urine-based HR-HPV testing is increasingly important [15]. This pilot study examining HR-HPV detection in a referral population had a number of important findings. First, HR-HPV prevalence was similar in first void urine collected at home in the morning, compared to that of initial stream and mid-stream urine collected later in the day. Second, unfractionated, pellet, and supernatant fractions of the first void and initial stream urine yielded similar HR-HPV prevalence estimates; however, mid-stream urine supernatant fractions had lower HR-HPV prevalence estimates than unfractionated and pellet urine fractions. Third, sensitivity, specificity, PPV, and NPV estimates of CIN2+ detection by HR-HPV DNA testing of urine were similar to respective values obtained for HR-HPV mRNA testing in both physician- and self-collected brush specimens. Lastly, urine collection was highly acceptable and preferred to brush self-collection among participating women.

To our knowledge, only one other pilot study among 10 Belgian women has compared HR-HPV detection in different urine samples within the same population of women [10]. Our results showing no difference in HR-HPV detection for first void, initial stream, and mid-stream urine for unfractionated and pellet fractions are in contrast to those from the Belgian study that found higher HR-HPV DNA detection in initial stream than in mid-stream samples [10]. This difference in findings is likely due to variations in laboratory procedures such as DNA extraction, amplification and detection. This Belgian study also found lower HR-HPV DNA detection in supernatant urine than in pellet urine fractions, which is consistent with our finding of lower HR-HPV DNA prevalence in mid-stream supernatant fractions than in unfractionated or pellet fractions (Table 2).

A recent meta-analysis comparing HR-HPV detection in urine versus cervical scrapes found higher sensitivity and specificity for HR-HPV detection in eight studies using first void urine samples compared to four studies using random or mid-stream urine samples [8]. Interpretation of these meta-analysis results remains unclear as some studies defined "first void" as the first urine sample of the day while others defined it as an initial stream collected later in the day. Furthermore, variations in findings from the meta-analysis may be due to differences in study populations and laboratory procedures among studies examined.

To our knowledge, our pilot study is the first to examine HR-HPV DNA detection in first void samples collected from first urination in the day compared to initial stream urine collected later in the same day from the same women. Since the interval from previous urination is likely to be longer with first void samples, it has been hypothesized that more exfoliated cells and subsequently more HR-HPV DNA may be obtained from first void as compared to initial stream urine collected later in the day [16]. In contrast to this hypothesis, our study found no difference in HR-HPV DNA in first void versus initial stream urine for any of the fractions tested per our collection protocol, suggesting that initial stream urine collected later in the day may be equally suitable for HR-HPV DNA detection. These findings have high clinical relevance, as the use of initial stream samples allows for sample collection similar to current practice for *Chlamydia trachomatis* or *Neisseria gonorrhoeae* testing, which requires an interval of only 1.5 h since last urination and does not restrict the time of day for urine collection [17].

The high sensitivity for CIN2+ detection by HR-HPV DNA testing in urine (90%) observed in our study is consistent with findings from another pilot study of 72 colposcopy patients that found sensitivity (92%) for CIN2+ detection with a prototype Trovagene urine-based assay [12]. These relatively high sensitivity estimates may be due to the preservation of DNA in the urine sample by inhibition of nucleases and the relatively small amplicon size. Similar studies conducting urine HPV testing among colposcopy patients using different HPV assays have found varying results. Song et al. found sensitivities of 70.8% and 62.8% for women with cervical carcinoma and CIN (grade unspecified), respectively, using a PCR-based DNA microarray chip system (Biomedlab, Co.; Seoul, Korea) [18]. Sellors *et al.*, found sensitivity of 44.8% for CIN2+ detection using the Hybrid Capture 2 (HCII) assay (QIAGEN, Gaithersburg, MD) [19].

Specificity for <CIN2+ detection from urine in this study (34%) was low, but comparable to specificity of HR-HPV mRNA testing for physician-collected (42.4%) and self-collected specimens (46.2%),

Table 2
Cervical Cancer Screening test results for 37 women attending a colposcopy clinic, stratified by participant histology.

Cervical cancer screening test[e]	Histology[a]		
	CIN2+ (N = 11)	<CIN2 (N = 26)	Overall (N = 37)
HR-HPV detection in unfractionated urine[b]			
First void sample			
Positive	9 (81.8%)	15 (57.7%)	24 (64.9%)
Negative	2 (18.2%)	11 (42.3%)	13 (35.1%)
Initial stream sample			
Positive	10 (90.9%)	17 (65.4%)	27 (73.0%)
Negative	1 (9.1%)	9 (34.6%)	10 (27.0%)
Mid-stream sample			
Positive	10 (90.9%)	16 (61.5%)	26 (70.3%)
Negative	1 (9.1%)	10 (38.5%)	11 (29.7%)
HR-HPV detection in pellet urine fractions[b]			
First void sample			
Positive	9 (81.8%)	16 (61.5%)	25 (67.6%)
Negative	2 (18.2%)	10 (38.5%)	12 (32.4%)
Initial stream sample			
Positive	10 (90.9%)	19 (73.1%)	29 (78.4%)
Negative	1 (9.1%)	7 (26.9%)	8 (21.6%)
Mid-stream sample			
Positive	10 (90.9%)	17 (65.4%)	27 (73.0%)
Negative	1 (9.1%)	9 (34.6%)	10 (27.0%)
HR-HPV detection in supernatant urine fractions[b]			
First void sample			
Positive	10 (90.9%)	17 (65.4%)	27 (73.0%)
Negative	1 (9.1%)	9 (34.6%)	10 (27.0%)
Initial stream sample			
Positive	11 (100.0%)	17 (65.4%)	28 (75.7%)
Negative	0	9 (34.6%)	9 (24.3%)
Mid-stream sample			
Positive	9 (81.8%)	12 (46.2%)	21 (56.8%)
Negative	2 (18.2%)	14 (53.8%)	16
Self-collection for HR-HPV detection[c,d]			
Positive	8 (80.0%)	13 (52.0%)	21 (56.8%)
Negative	2 (20.0%)	12 (48.0%)	14 (37.8%)
Physician-collection for HR-HPV detection[c]			
Positive	10 (90.9%)	15 (57.7%)	25 (67.6%)
Negative	1 (9.1%)	11 (42.3%)	12 (32.4%)

[a] CIN2+ = cervical intraepithelial neoplasia Grade 2 or more severe. Women with no visible lesions at colposcopy were categorized as disease negative (<CIN2) for data analyses. Directed biopsies were performed in women with visible lesions to determine the histological grade of the lesions (CIN2+ or <CIN2).
[b] Trovagene test used for HR-HPV DNA detection in all urine testing.
[c] Aptima assay used for HR-HPV mRNA detection in self- and physician-collected brush samples.
[d] Two participants had invalid HR-HPV test results for self-collected samples.
[e] Numbers and percentages may not sum up to totals due to missing values and rounding.

yet somewhat lower than estimates from the Sellors et al. study (69.7%) 19].

Strengths of our study include the ability to compare HR-HPV detection within and across three different urine sample types and three different urine fractions, and to mRNA detection in physician- and self-collected genital specimens for CIN2+ detection for each patient. This study provides information on reported acceptability of and preference for urine versus brush self-collection, and of delivery of urine collection kits by mail, which could inform future implementation. Study limitations include the lack of a HR-HPV DNA comparison test on the genital specimens collected in this study; however, the Aptima HPV assay has been repeatedly shown to have high sensitivity and specificity for CIN2+ detection [20,21]. Kappa values for agreement in HR-HPV detection between urine samples were moderate; this is likely due to the fairly high HR-HPV prevalence and moderate prevalence index that can lower kappa estimates [22]. Our study was conducted in a high-risk colposcopy population, with a small sample size. These findings for the validity of the Trovagene HPV HR test need to be confirmed in a larger study of a general screening population.

This pilot study found that HR-HPV DNA detection in urine by the Trovagene HPV HR test in three different fractions from first void and initial stream urine was highly sensitive for the detection of CIN2+ in colposcopy patients. Though further validation studies are needed in larger population-representative samples of women, findings suggest that HR-HPV testing via non-invasive, simple, and highly acceptable urine sampling may be a viable method for cervical cancer screening, particularly in hard-to-reach populations or in limited-resource settings.

Funding

This research was supported by a grant from Trovagene Inc. to the Department of Epidemiology at the University of North Carolina at Chapel Hill. Hologic Inc. provided collection media and HR-HPV mRNA testing for self- and physician-collected genital specimens, and Rovers Medical Devices provided brushes for self- and physician-collected genital specimens.

Conflict of interest

V. Senkomago has received travel support from Hologic Inc. over the past year, and A. Des Marais has received travel support from Hologic Inc. over the past 3 years. J.S. Smith has received consultancy and/or research grants from Hologic Inc, BD Diagnostics, Trovagene, and QIAGEN over the past five years. C.R.T. Vibat, and M.G. Erlander are employees of Trovagene. No other coauthors have conflicts of interest to report. Dr. Rahangdale has received research support from Hologic, Inc. and QIAGEN over the last 5 years.

Ethics approval

This study was approved by the Institutional Review Board at the University of North Carolina at Chapel Hill, NC.

Acknowledgments

We would like to thank the women who volunteered to participate in this study. The authors are grateful to Iesha Williams, Laura Baker, and Kathleen McClain for their assistance in data collection, Eugenio Nunez and Adriana Muniz for conducting HR-HPV laboratory testing in urine and managing lab data, Lanya Shapiro and Chris Wiesen for their assistance in data cleaning and database management, and Janel Dockter for her critique and editing of the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcv.2015.11.005.

References

[1] L. Gustafsson, J. Pontén, M. Zack, H. Adami, International incidence rates of invasive cervical cancer after introduction of cytological screening, Cancer Causes Control 8 (1997) 755–763.
[2] E. Gakidou, S. Nordhagen, Z. Obermeyer, Coverage of cervical cancer screening in 57 countries: low average levels and large inequalities, PLoS Med. 5 (2008) e132.
[3] W.A. Leyden, M.M. Manos, A.M. Geiger, S. Weinmann, J. Mouchawar, K. Bischoff, et al., Cervical cancer in women with comprehensive health care access: attributable factors in the screening process, J. Natl. Cancer Inst. 97 (2005) 675–683.
[4] V.B. Benard, C.C. Thomas, J. King, G.M. Massetti, V.P. Doria-Rose, M. Saraiya, Vital signs: cervical cancer incidence, mortality, and screening—United States, 2007–2012, MMWR Morb. Mortal Wkly Rep. 63 (2014) 1004–1009.

[5] E.J. Nelson, J. Hughes, J.M. Oakes, B. Thyagarajan, J.S. Pankow, S.L. Kulasingam, Human Papillomavirus Infection in women who submit self-collected vaginal swabs after internet recruitment, J. Community Health 40 (2015) 379–386.
[6] D.E. Stewart, A. Gagliardi, M. Johnston, R. Howlett, P. Barata, N. Lewis, et al., Self-collected samples for testing of oncogenic human papillomavirus: a systematic review, J. Obstet. Gynaecol. Can. 29 (2007) 817–828.
[7] E. Enerly, C. Olofsson, M. Nygård, Monitoring human papillomavirus prevalence in urine samples: a review, Clin. Epidemiol. 5 (2013) 67.
[8] N. Pathak, J. Dodds, J. Zamora, K. Khan, Accuracy of urinary human papillomavirus testing for presence of cervical HPV: systematic review and meta-analysis, BMJ 349 (2014) g5264.
[9] A. Vorsters, I. Micalessi, J. Bilcke, M. Ieven, J. Bogers, P. Van Damme, Detection of human papillomavirus DNA in urine: a review of the literature, Eur. J. Clin. Microbiol. Infect. Dis. 31 (2012) 627–640.
[10] A. Vorsters, J. Van den Bergh, I. Micalessi, S. Biesmans, J. Bogers, A. Hens, et al., Optimization of HPV DNA detection in urine by improving collection, storage, and extraction, Eur. J. Clin. Microbiol. Infect. Dis. 33 (2014) 2005–2014.
[11] Trovagene HPV testing urine-based HPV test, Available at http://www.trovagene.com/our-tests/hpv/ 2014; 2015.
[12] V.V. Sahasrabuddhe, P.E. Gravitt, S.T. Dunn, D. Robbins, D. Brown, R.A. Allen, et al., Evaluation of clinical performance of a novel urine-based HPV detection assay among women attending a colposcopy clinic, J. Clin. Virol. (2014).
[13] Hologic Inc. APTIMA/HPV Assay [Package Insert]. Bethseda, MD: Hologic Inc.; 2008.
[14] K.J. Rothman, S. Greenland, T. Lash, Median unbiased estimates Anonymous Modern Epidemiology, vol. 221, 3rd ed., Lippincott Williams & Wilkins, Philadelphia, PA, 2008, pp. 255–256.
[15] W.K. Huh, K.A. Ault, D. Chelmow, D.D. Davey, R.A. Goulart, F.A. Garcia, et al., Use of primary high-risk human papillomavirus testing for cervical cancer screening: interim clinical guidance, Gynecol. Oncol. (2015).
[16] A. Vorsters, D. Van, P. amme, G. Clifford, Urine testing for HPV: rationale for using first void, BMJ 349 (2014) g6252.
[17] Centers for Disease Control. Laboratory Procudeure Manual. Analyte: Chlamydia trachomatis and Neisseria gonorrhoeae. Matrix: Urine. 2007; 2014, 30.
[18] E.S. Song, H.J. Lee, T.S. Hwang, Clinical efficacy of human papillomavirus DNA detection in urine from patients with various cervical lesions, J. Korean Med. Sci. 22 (2007) 99–104.
[19] J.W. Sellors, A.T. Lorincz, J.B. Mahony, I. Mielzynska, A. Lytwyn, P. Roth, et al., Comparison of self-collected vaginal, vulvar and urine samples with physician-collected cervical samples for human papillomavirus testing to detect high-grade squamous intraepithelial lesions, CMAJ 163 (2000) 513–518.
[20] J. Monsonego, M.G. Hudgens, L. Zerat, J. Zerat, K. Syrjänen, P. Halfon, et al., Evaluation of oncogenic human papillomavirus RNA and DNA tests with liquid-based cytology in primary cervical cancer screening: The FASE study, Int. J. Cancer 129 (2011) 691–701.
[21] M.H. Stoler, T.C. Wright, J. Cuzick, J. Dockter, J.L. Reid, D. Getman, et al., APTIMA HPV assay performance in women with atypical squamous cells of undetermined significance cytology results, Obstet Gynecol 208 (2013) 144, e1, 144. e8.
[22] J. Sim, C.C. Wright, The kappa statistic in reliability studies: use, interpretation, and sample size requirements, Phys. Ther. 85 (2005) 257–268.