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

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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.

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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

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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].

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