Vaginal Swab Measurement of Human Papillomavirus in Wave I of the Social Life Health & Aging Project

Authors:

Stacy Tessler Lindau, MD, MAPP, University of Chicago, Departments of Ob/Gyn and Medicine – Geriatrics*

Hanna Surawska, BA, University of Chicago, Department of Ob/Gyn

Jessica Schwartz, BA, University of Chicago, Department of Ob/Gyn

Jeanne A. Jordan, PhD, University of Pittsburgh, Department of Pathology, Magee-Women's Research Institute

* Corresponding author. Fax: +1 773 834 5664.
E-mail address: slindau@uchicago.edu (S.T. Lindau).

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Rationale

Human papillomavirus (HPV), a common sexually transmitted viral infection of the vagina and cervix, is an important factor in genital tract dysplasia (precancer) and is found in association with nearly all cases of cervical cancer (Schiffman, Bauer et al. 1993). More than 100 types of HPV have been identified, with as many as 40 infecting the female genital tract (Munoz, Bosch et al. 2003). HPV infection in younger women is frequently transient but can be persistent, latent, or reactivated. Risk factors for genital tract HPV in women include sexual activity, tobacco use, and immune suppression(Bosch, Lorincz et al. 2002). NSHAP provides data on the presence and subtypes of high risk HPV, or the strains that are most strongly associated with cervical dysplasia and cancer. Genital tract HPV presence may be a marker of immune function in older women (Garcia-Pineres, Hildesheim et al. 2006).

Measurement

High-risk (HR) HPV DNA testing was performed using the HC2 assay method (Digene Corp.) and rapid capture system (RCS) according to the manufacturer's protocol. The HR HC2 signal amplification assay contains a cocktail of probes complementary to 13 HR HPV types including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. In addition to the kit controls, an external sample processing control consisting of approximately 5,000 HeLa cells, which contain HPV 18 DNA was processed with each batched of specimens to control of adequate cell lysis. Specimens with Relative Light Unit/Cutoff Values equal to or greater than 1.0, obtained using the DML 2000 instrument were considered positive for HR-HPV DNA.(Lindau and Drum Under Review)

Population Prevalence

No population-based estimates are available for older women in the U.S.; however, clinical studies of post-menopausal women suggest that 10-20% have any genital tract HPV infection. Research outside the U.S. suggests that prevalence of high-risk subtypes is lower, ranging from 5.8% cumulative frequency of HPV-HR positivity among women 55 and older (Smith, Ritchie et al. 2003) to 13.6% for oncogenic subtypes for women age 65+ (Herrero, Castle et al. 2005)

Table 1: Human Papillomavirus Prevalence in Older Women (%)

	Rates of HPV Positivity*	
Age	***Smith et al. (2004)***	***Smith et al. (2003)***
<55	13.0	13.5
55-59	20.3	15.7
60-65		12.4
65+	N/A	

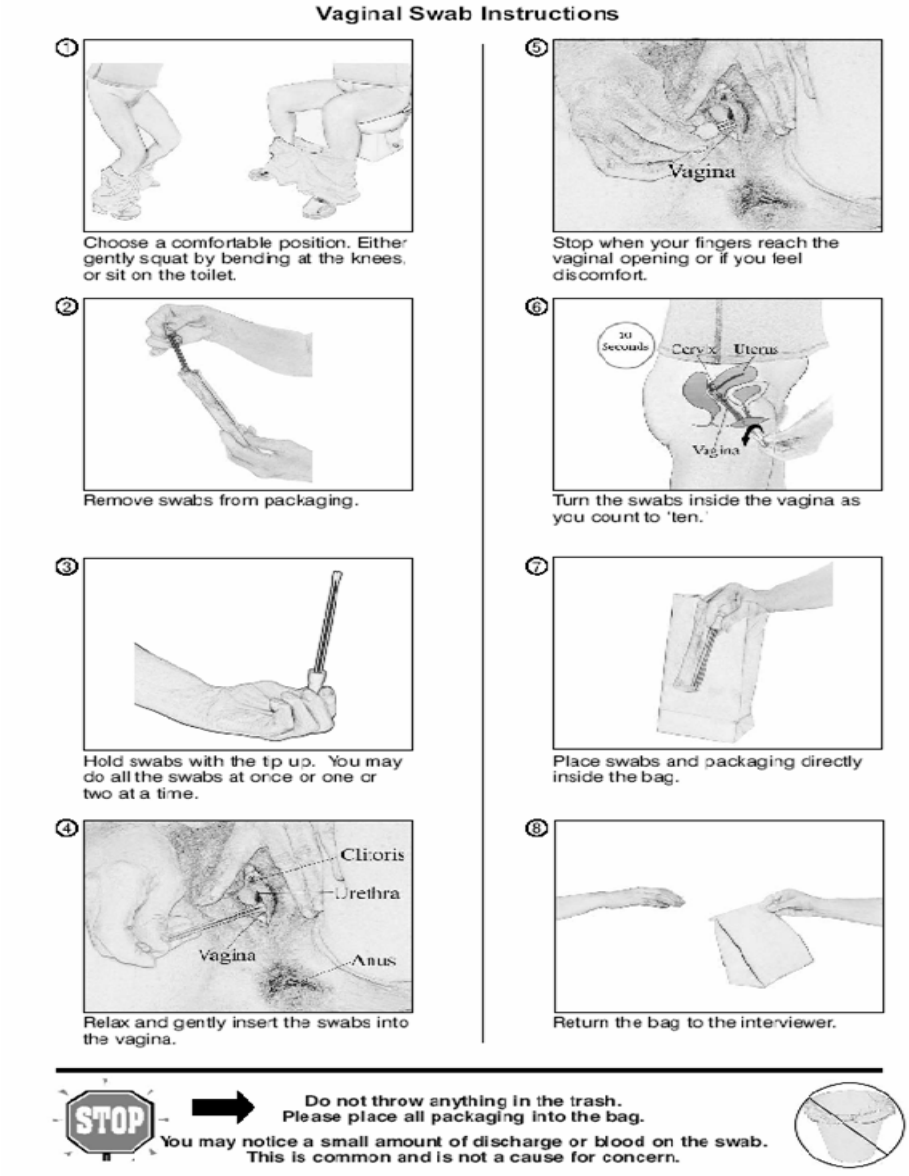
**HPV positivity includes both high-risk and low-risk subtypes*

Specimen Collection (Females Only)

The Vaginal Swab Protocol was designed to collect vaginal specimens for Bacterial Vaginosis (BV), Vaginal Candidiasis (VC), Human Papillomavirus (HPV) testing, and vaginal cytology analysis. All female respondents were asked to provide a vaginal self-swab specimen. Procedures were explained using a scripted description aided by illustrated instructions, developed for NSHAP by a medical illustrator in conjunction with a study investigator who is a gynecologist (Figure 1). Participant questions were addressed using a "frequently asked questions" document to ensure consistency of responses across field staff. Field staff read each step of the illustrated instructions to the respondent and asked for questions. Participants were given the instruction card with the collection materials (Female Swab Specimen Collection Kit, Catalog No. 5123-1220; Digene Corporation, Gaithersburg, MD and BBL™ CultureSwab™ Plus, Catalog No. 220117; Becton, Dickinson and Company, Franklin Lakes, NJ) and directed to a

bathroom or other private room in the home. When the respondent returned, the interviewer then secured the Digene swab inside a tube containing 1 mL Specimen Transport Medium™ (STM; Digene Corp.) and the BBL™ CultureSwab™ inside a tube containing Amies medium without charcoal (Becton, Dickinson and Company). The interviewer labeled both tubes with the unique, numeric identification number. At the end of each home encounter, field staff stored the vaginal swab transport tubes in an insulated cooler with ice packs. Vaginal swabs were shipped daily on cold packs in a Styrofoam container to the University of Pittsburgh, Magee-Women's Hospital Department of Pathology clinical microbiology laboratory via overnight delivery. The swabs were packaged in accordance with the federal shipping guidelines for diagnostic biological material. Following processing at Magee-Women's, one BBL™ CultureSwab™ for each respondent was repackaged and shipped overnight on cold packs to the University of Chicago Institute for Mind and Biology laboratory for cytological analysis. An interactive reconciliation system facilitated remote tracking of vaginal swabs. Vaginal swab specimens were collected from all willing female respondents (n = 1,028), with an adjusted cooperation rate of 67.6%

Figure 1. Self Collection of Vaginal Epithelial cells using Dacron Swab



Instructions developed by medical illustrator Rachel Seelen in conjunction with Stacy Lindau, MD.

Shipping and Storage

After collection, the vaginal swab tubes were given to the interviewer and stored at 2- 8°F, in an insulated bag with two reusable ice packs. Specimens were shipped daily.

Prior to shipping, collection tubes containing specimens were removed from the insulated bag, placed in a small Ziploc bag with a handful of cotton balls, and then placed in a Styrofoam™ container and 8" x 7" x 7" cardboard box with a disposable ice pack. They were shipped to The University of Pittsburgh Department of Pathology, Magee-Women's Hospital Clinical Microbiology Laboratory by FedEx Express, by placing the package in a drop box or calling for pickup by FedEx.

Method	FedEx Express: Placed in drop box or picked up by FedEx
Shipping Address	Jeanne Jordan Magee-Women's Hospital Clinical Microbiology Lab 300 Halket Street, Room 4680 Pittsburgh, PA 15213 (412) 641-4104

Assay

Table 2: NSHAP Vaginal Swab assay used by Magee-Women's Research Institute	
	Digene Hybrid Capture 2 (hc2) HPV DNA Test
Assay principle	In *vitro* nucleic acid hybridization assay with signal amplification using microplate chemiluminescence
Intended use	Qualitative detection of 13 high risk HPV types
Regulatory status	FDA Approved
Analytical Sensitivity	1.09 pg/mL [0.97-1.27 pg/mL]
Cross Reactivity	HPV 13, 6, 42

High-risk HPV Detection by HC2 Assay

High-risk (HR) HPV DNA testing was performed using the HC2 assay method (Digene Corp.) and rapid capture system (RCS) according to the manufacturer's protocol. The HR HC2 signal amplification assay contains a cocktail of probes complementary to 13 HR HPV types including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. In addition to the kit controls, an external sample processing control consisting of approximately 5,000 HeLa cells, which contain HPV 18 DNA was processed with each batched of specimens to control of adequate cell lysis. Specimens with Relative Light Unit/Cutoff Values equal to or greater than 1.0, obtained using the DML 2000 instrument were considered positive for HR-HPV DNA. Both a negative control and a high-risk HPV calibrator are included in the kit. Each control is tested in triplicate within each run. For the run to be valid, the negative control %CV must be at or below 25%, and the calibrator %CV must be at or below 15%.

HPV Genotyping Test

HPV genotyping steps were carried out on all HC2 HR-HPV positive specimens using the Linear Array HPV Genotyping Test and Linear Array Detection Kit (Roche Molecular Systems Inc., Pleasanton, CA) according to the manufacturer's instructions. The Linear Array HPV Genotyping Test is intended for research use only, and not for use in diagnostic procedures. To be compatible with the Qiagen MinElute Spin Column protocol for DNA extraction (Cat. # 57704, Qiagen Inc. Valencia, CA), 250 microliters of each denatured STM was re-acidified to a pH just below 7.0 prior to DNA purification. Fifty microliters of the 120 microliters elution volume were added to an equal volume of 2X PCR master mix containing hot-start *Taq polymerase* and biotin-labeled PGMY09/11 primer set to amplify 37 different anogenital HPV genotypes; They include the following HR-HPV and low-risk types of HPV: 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39 and CP6108. The HPV target is approximately 450 base pairs in length. In the same PCR reaction, a 268 bp target of the human beta-globin genes is co-amplified and served as an internal control for DNA amplification. Thermocycling was performed using the Applied Biosystems Gold-plated GeneAmp^{R} 9700 thermocycler. Amplicon denaturation and detection was carried out using the Linear Array Detection Kit according to the manufacturer's directions. External known negative and known positive specimens were included in each run. Two independent readers interpreted the results. Discordant results were re-reviewed by the initial two readers to resolve any discrepancy.

Quality Control

The laboratory participates in semi-annual proficiency testing for High-risk HPV testing using the HC2 assay, and must achieve greater than 90% overall agreement with these challenges.

Reproducibility Study

A total of 20 STM-based specimens (10-positive and 10 negative) were tested in replicates of four on each of five days, for a total of 20 replicates. According to the manufacture's package insert, STM-based specimens with a mean RLU/CO of 20% or more above the cutoff were positive 100% of the time, and those with a mean RLU/CO of 20% or less below the cutoff were negative 100% of the time. Those specimens that were close to the cutoff yielded approximately equal numbers of positive and negative results.

Effect of potentially interfering substances of STM specimens: The effects of 4 agents were studied (blood, douche, anti-fungal cream and contraceptive jelly). None of the 4 agents added to STM specimens resulted in any false positive results. It was noted that a false negative result could occur with specimens containing low levels of high-risk HPV DNA (approximately 1 pg/ml) in the presence of high concentrations of anti-fungal cream or contraceptive jelly.

Analytical Sensitivity

The manufacturer established the analytical sensitivity using plasmid DNA. The mean limit of detection for all 13 types of HPV was 1.08 pg/ml (95% CI= 0.95-1.25, with a SD = 0.05).

Cross Reactivity

According to the manufacturer cross reactivity occurs with HPV type 6, and 42 when present at concentrations above or at 4 ng/ml. Cross reactivity has also been reported for HPV types 40, 53 and 66.

Availability

	STM Vaginal Swab	Blue-Tipped Vaginal Swab (double swab)
Product Name	Female Swab Specimen Collection Kit *Collection kit includes: sterile Dacron® swab and tube with 1 mL of Specimen Transport Medium™	BD BBL™ CultureSwab™ Plus Amies without Charcoal
Manufacturer	Digene Corporation	Becton, Dickinson and Company
Location of Manufacturer	Gaithersburg, MD	Franklin Lakes, NJ
Product Number	5123-1220	220117
Interviewer Instructions	When respondent returns, the interviewer inserts the swab into the STM tube and breaks off the extra handle of the swab by pressing it along the side of the tube. The interviewer then labels the tube with the respondent's SUID number using a Sharpie pen and a blank lab label.	When respondent returns, remove cap from tube with gel and tightly insert blue tipped swab in gel. Label tube with Respondent's SUID number using a Sharpie pen and a blank lab label.

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