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Vaginal Self-Swab Specimen Collection in a Home-Based Survey of Older Women: Methods and Applications

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Objectives. To describe the methods used for, cooperation with, assays conducted on, and applications of vaginal specimens collected by older women in their homes.

Methods. Community-residing women ($N = 1,550$), ages 57–85 years, participated in a nationally representative probability survey. Vaginal self-swab specimen collection and in-home interviews were conducted between 2005 and 2006. Specimens were analyzed for bacterial vaginosis (BV), vaginal candidiasis (VC), high-risk human papillomavirus (HR-HPV), and cytological characteristics. Field methods, consent procedures, the swab protocol, laboratory procedures, and results reporting are described.

Results. One thousand twenty-eight respondents (67.5% weighted) agreed to provide a vaginal specimen; 99.1% were successful. The specimen adequacy rates were BV and VC, 94.1%; HR-HPV, 99.7%; and cytology, 85.5%. The most common recorded reason for nonparticipation was a physical or health problem (38% of nonresponders). Responders were significantly more likely than nonresponders to be younger and more educated, and were more likely to report a recent pelvic examination, menopausal hormone use, and recent sexual activity.

Discussion. Collection of vaginal self-swab specimens from older women in a population-based study is feasible and provides novel data on microenvironmental characteristics of the female genital tract relevant to analyses of gynecologic health, sexual activity and problems, and immune and inflammatory function.

Key Words: Vaginal swab—Vaginal cytology—Self-collection—Methods—Protocol—Older women—Population.

WOMEN ages 60 years and older make up a large and growing portion of the United States population and are projected to outnumber men at older ages through 2050 (U.S. Census Bureau, 2004). For men, aging is largely a partnered experience. The majority of men experience aging, illness, and death with their life partner. In contrast, many women experience their later years alone (Lindau et al., 2007). The implications of these gender differences for health in later life are poorly understood. The National Social Life, Health, and Aging Project (NSHAP) was designed, in part, to identify biological mechanisms through which social, including intimate and sexual, relationships affect health as women and men age.

Collection of sensory function measures (olfaction, gustatory function, vision, tactile sensation) (Schumm et al., in review), salivary sex hormone measures (estrogen, testosterone, progesterone, dehydroepiandrosterone) (Gavrilova & Lindau, in review), and vaginal mucosal measures in a single, population-based study of older adults is novel to NSHAP and reflects the study's orientation toward understanding the physiology of social and sexual relationships and a focused interest in addressing largely neglected areas of older women's health.

Here, we provide the initial detailed description of the rationale and methods for vaginal self-swab specimen collection in the NSHAP study, as well as applications of these data for health research. Design and implementation of the vaginal self-swab protocol including transportation, methods for selecting, training, and supporting field staff in vaginal swab collection, laboratory procedures and coordination, and a results reporting system are described. Specimens were analyzed for bacterial vaginosis (BV), vaginal candidiasis (VC) or yeast, high-risk human papillomavirus (HR-HPV), and cytological characteristics. Participant cooperation, specimen adequacy, and explanations of missing data are reported.

The first wave of NSHAP, conducted between July 2005 and March 2006, included collection of vaginal specimens from female respondents. Vaginal specimens provide microbiologic material, including fungal, viral, and bacterial pathogens that can be deleterious for women's health and functioning. The rationale for the assays (biological

analyses) performed on the NSHAP vaginal swab specimens, including BV, VC or yeast, human papillomavirus, and vaginal cellular characteristics (cytology), follows.

BV is a common polymicrobial condition of the vagina that occurs with reduction of vaginal acidity (pH) and a shift in the normal bacteria that colonize the vagina. The estimated population prevalence of this condition among younger women in the United States (ages 14–49 years) is 29% (Allsworth & Peipert, 2007). While often asymptomatic, symptoms include a thin, fishy vaginal discharge that can worsen following sexual intercourse. Douching, sexual intercourse with multiple partners, oral sex particularly with a new sexual partner, and cigarette smoking are associated with BV in younger populations (Allsworth & Peipert, 2007; Marrazzo et al., 2002; Ness et al., 2002; Smart, Singal, & Mindel, 2004). BV has been associated with infection of the lining of the uterus and upper genital tract, urinary tract infections, infectious complications following gynecologic procedures, and sexually acquired HIV transmission (Harmanli, Cheng, Nyirjesy, Chatwani, & Gaughan, 2000; Peipert, Montagno, Cooper, & Sung, 1997; Sewankambo, 1997; Soper, Bump, & Hurt, 1990). Although most research on BV has been conducted in younger populations, one northern Italian study of 921 postmenopausal women seeking gynecologic care found a BV prevalence of about 6% (Cauci et al., 2002). This is likely a low estimate of the population prevalence of BV among older women, as the condition may inhibit women from seeking gynecologic services or be associated with other barriers to medical care. BV can be persistent, irritating, and malodorous, and left untreated can compromise quality of life, including sexual activity and functioning.

VC results from overgrowth of a yeast species commonly found in the normal vaginal flora. It is highly prevalent among younger women and, based on clinical data, is less frequently diagnosed among postmenopausal women. Yeast can cause vaginal and vulvar irritation, itching, burning with urination, and a thick white vaginal discharge. It can be episodic, recurrent, or chronic and, if not treated, can cause disruption in sexual and social functioning due primarily to the physical discomfort. Obesity, incontinence, and sedentariness can promote and/or exacerbate yeast vaginitis. The symptoms are similar to those, and must be distinguished from, thinning of the vaginal epithelium (atrophy) that occurs with loss of estrogen in postmenopausal women. Yeast vaginitis can be sexually transmitted or associated with sexual activity but commonly occurs in women who are not sexually active. Risk factors in older women include diabetes mellitus, immune suppression, and use of antibiotics, estrogen, and pessaries (vaginal devices used to treat pelvic organ prolapse) (Sobel et al., 1998).

Human papillomavirus (HPV), a common sexually transmitted viral infection of the anogenital tract, is an important factor in genital tract dysplasia (precancer) and high risk types are found in association with nearly all cases of cervical cancer. More than 100 types of HPV have been identified, with as many as 40 infecting the female genital tract. 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 exposure, and immune suppression (Baseman & Koutsky, 2005). NSHAP provides data on the presence and subtypes of HR-HPV or the strains that are most strongly associated with cervical dysplasia and cancer. With recent availability of an assay for identification of low-risk HPV subtypes (associated with vulvar and vaginal warts), work is under way to make these data available from the NSHAP specimens. For the first time, these data provide information on population prevalence and correlates of HPV among older women in the United States (Lindau, Drum, Gaumer, Surawska, & Jordan, in press). Genital tract HPV presence may be a marker of immune function in older women (Garcia-Pineres et al., 2006).

The vaginal swab specimens also provide cellular material useful for gauging sex hormone metabolism and, in particular, the bioactivity of estrogens within the vaginal epithelium. The vaginal epithelium is the cellular layer that lines the vagina and is physiologically important for sexual function and an indicator of the overall integrity of the female urogenital tract, including the bladder, urethra, vagina, vulva, and pelvic floor support structures. To date, population data have not been available for documentation of the cytological features of the vagina in older women, particularly in relation to sexual function. These data are important for basic scientific understanding of older female endocrine physiology and may relate to sexual function and behavior in later life.

These measures and possible applications are summarized in Table 1.

Vaginal self-swab methods have been used in previous studies of younger women, mainly to assess HPV prevalence or as an alternative to the conventional clinical Pap smear procedure (Dunne et al., 2007). These methods have been particularly useful in remote and resource-poor settings. Women are receptive to these methods, find them acceptable, and, in some cases, prefer self-swab to gynecologic examination (Chernesky et al., 2005). Few studies have used vaginal self-swab procedures with older women. NSHAP worked with investigators for the National Health and Nutrition Examination Survey (NHANES) to harmonize the vaginal self-swab protocols; some data from younger women in NHANES are therefore comparable to data from older women in NSHAP. To the best of our knowledge, NSHAP is the first study to use a vaginal self-swab protocol with a large, home-based cohort of older women.

Methods

Details of the NSHAP sample design and participant characteristics have been previously described (Lindau et al., 2007). Here, we describe the translation and adaptation

Table 1. Summary of Vaginal Self-Swab Measures and Applications

	Definition	Acquisition	Relevance	Biomeasure Code Interpretation
BV	Shift in vaginal flora with elevation in vaginal pH (reduction in vaginal acidity)	Not sexually transmitted but may be exacerbated by vaginal or oral sexual activity	Odorous discharge, vaginal irritation may interfere with sexual and social functioning; common gynecologic condition among younger women	Normal vaginal flora; intermediate vaginal flora; vaginal flora indicative of BV
VC	Overgrowth of vaginal yeast species with reduction in vaginal pH	Typically not sexually transmitted but can be passed between sexual partners via vaginal, penile, or oral reservoirs	Vaginal itching, irritation may interfere with sexual and social functioning; common gynecologic condition among younger women; may indicate underlying metabolic disease	Detectable yeast absent; detectable yeast present
HPV	Viral infection of genital tract associated with most precancers and cancers of the cervix and with genital tract condyloma (warts)	Sexually transmitted, but presence can indicate persistent, latent, or new infection. Assay cannot distinguish timing of infection.	Asymptomatic and communicable condition that can result in precancerous and cancerous genital tract changes	HR-HPV absent; HR-HPV present; presence of greater than or equal to 1 of 37 HPV anogenital tract genotypes
Vaginal cytology[a]	Microscopic observation of vaginal cellular characteristics, vaginal MI	Does not apply	Indicator of vaginal estrogen bioactivity and cumulative systemic estrogen status. Small relative proportion of superficial epithelial cells as indicated by MI may relate to vulvovaginal irritation and burning; may interfere with sexual and social functioning	Excellent for MI: ≥100 cells present. Adequate for MI: 50–99 cells present; Inadequate for MI: <50 cells present

Notes: BV = bacterial vaginosis; VC = vaginal candidiasis; HPV = human papillomavirus; HR-HPV = high-risk HPV; MI = maturation index.
[a]Coders not trained to identify cellular dysplasia or cytopathy.

of common clinical methods of in-home vaginal microbiological and cytological assessment.

Design and Implementation of the Vaginal Swab Collection Protocol

Human subjects protocol.—All NSHAP data are protected by approval from local Institutional Review Boards and by a National Institutes of Health (NIH)–issued Certificate of Confidentiality (National Institutes of Health, 2003). The vaginal swab informed consent protocol involved: (a) signed consent prior to initiating the interview, (b) detailed description of collection procedures followed by oral reconsent at the time of vaginal swab collection, and (c) for those providing a specimen, signed consent to receive a mailed notification of results availability. To ensure anonymity of the vaginal specimens, each was labeled with a unique identification number used for tracking in transportation, and processing, and the results reporting system was operated by the American Social Health Association (ASHA, Research Triangle Park, NC). ASHA has expertise in contact center operations and extensive experience in providing test results counseling services for federal research and clinical trials.

Laboratories and ASHA had access to only these unique identifiers. All the vaginal specimen data are available via the NSHAP public use data set, with the exception of the HPV assay results. Because a small proportion of the population tested positive for HPV and individuals may have disclosed their HPV status to sexual partners, these data are restricted to maximize protection of participant anonymity.

Selection, training, and support of field staff for vaginal swab collection.—The National Opinion Research Center (NORC) maintains a large, national field staff with extensive experience in survey interviewing. Interviewer recruitment and training protocols were tailored to support the breadth and nature of biological data collection unique to NSHAP, including vaginal swab collection. A detailed description of interviewer recruitment, staffing, and training as it relates to integrating biomeasures into a social science survey has been previously published (Jaszczak, Lundeen, & Smith, in review).

Field staff recruitment involved detailed description of expectations and tasks related to the vaginal swab protocol. Candidate willingness to handle and store vaginal swab materials and comfort discussing the protocol and its rationale with participants were assessed. Field staff training procedures combined NORC survey interviewing expertise with the clinical expertise (gynecology, internal medicine) of members of the investigator team. Vaginal swab training included: (a) pretraining home study using a customized training video and manual; (b) in-person training involving direct contact with study investigators (including a clinical gynecologist), active learning techniques, and practice–feedback sessions with trainers; and (c) phone-based small group booster trainings with NORC staff and a physician study investigator during the field period. All female NSHAP participants were interviewed by female field staff (the NORC field staff pool is predominantly female).

Vaginal swab specimen collection, processing, and results reporting.—All female respondents were asked to provide a vaginal self-swab specimen. Biomeasure collection

occurred midway through the interview to facilitate respondent comfort with the interviewer and the procedures. 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.

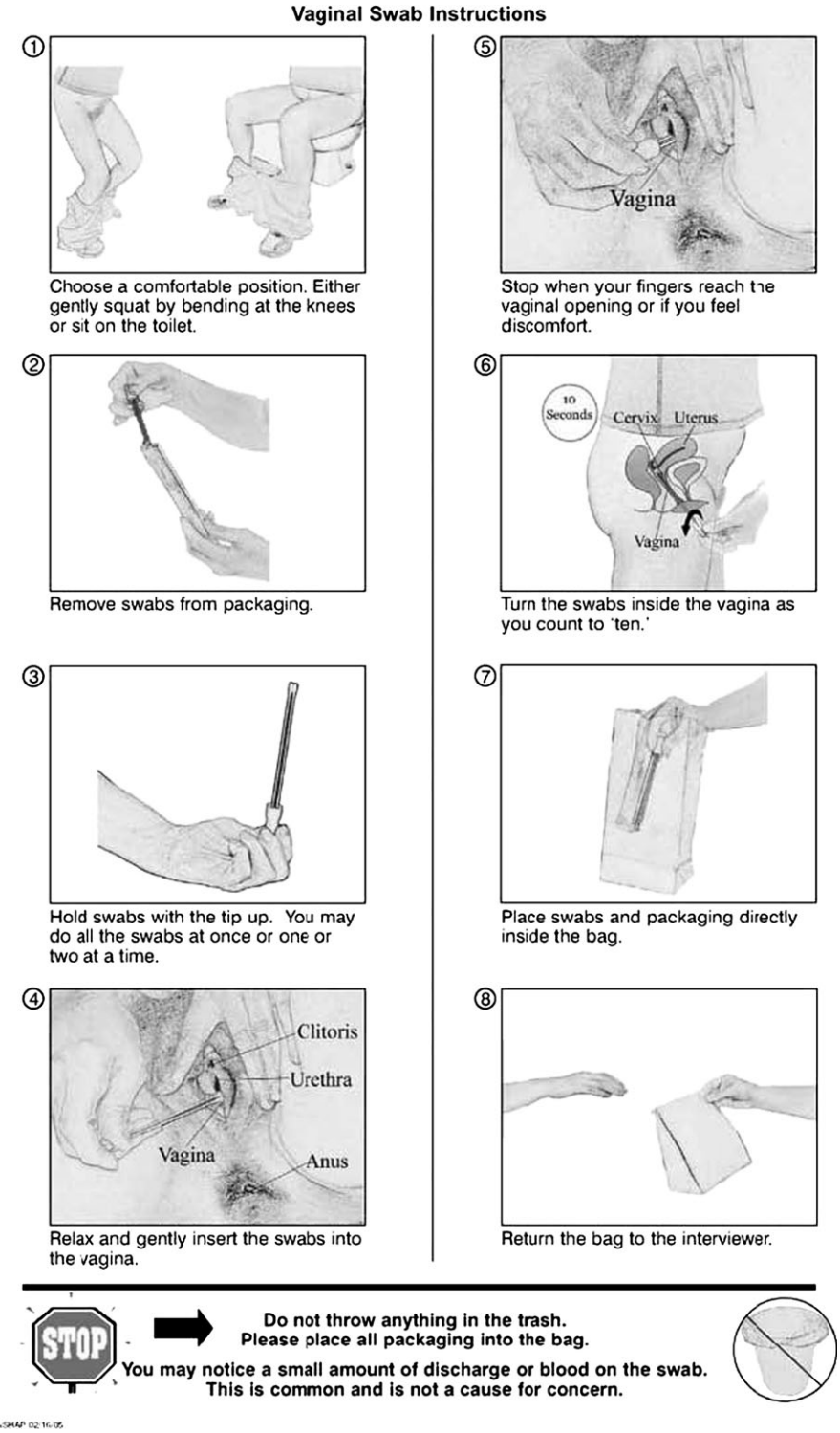

Figure 1. Illustrated instructions for self-collected vaginal swab by older women. Illustration by Rachel Seelen (Chicago, IL) in collaboration with Stacy Tessler Lindau.

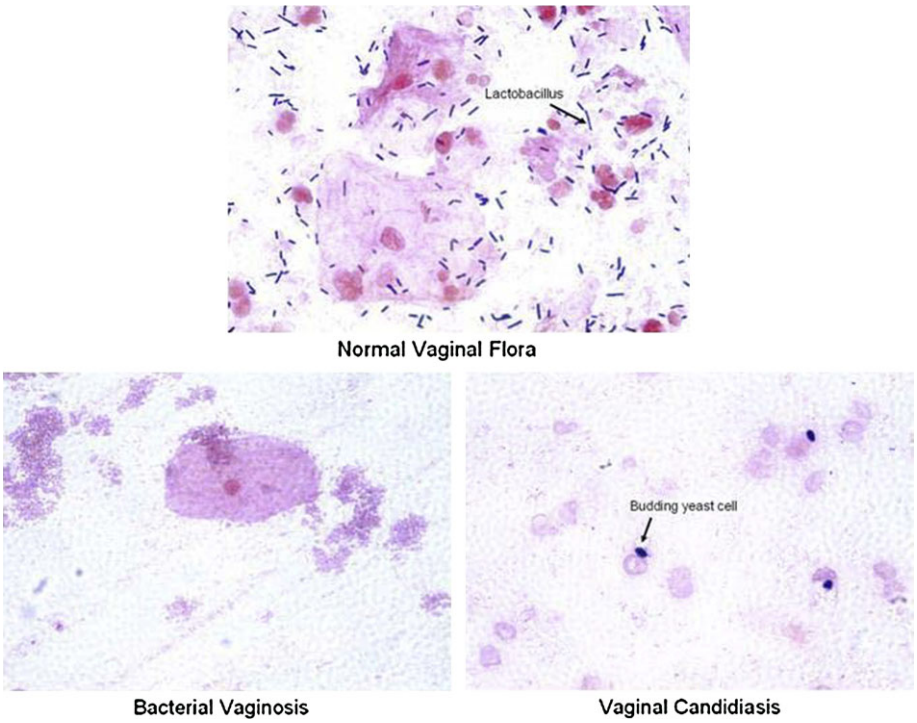

Figure 2. Normal vaginal flora, bacterial vaginosis (BV), and vaginal candidiasis (VC). Normal vaginal flora: A gram-stained vaginal swab–based sample that was given a score of 1, which is indicative of normal flora. The field contains epithelial cells with significant numbers of *Lactobacillus* spp. (arrow) but lacked small gram-negative rods (×1,000 magnification). BV: A gram-stained vaginal swab–based sample that was given a score of 10, which is indicative of BV. The field contains a single epithelial cell with significant numbers of small gram-negative rods and lacked any *Lactobacillus* spp. (×1,000 magnification). VC: A gram-stained vaginal swab–based sample. The field contains several gram-positive yeast cells, including one that is budding (arrow) (×1,000 magnification). Microscope: Nikon Eclipse E600; Code No. 2CE-MVZH-9; Nikon Corporation (Tokyo, Japan).

Participants were given the instruction card with the collection materials (details on materials have been previously described) and directed to a bathroom or other private room in the home (Lindau, Mendoza, Surawska, & Jordan, 2008a, 2008b, 2008c). When the respondent returned, the interviewer then secured the Digene swab inside a tube containing standard transport medium and the BBL™ CultureSwab™ inside a tube containing Amies medium without charcoal (Becton, Dickinson and Company, Franklin Lakes, NJ). The interviewer labeled both tubes with the unique, numeric identification number. Additional details regarding the protocol are summarized below and have been previously published (Lindau, Drum, Gaumer, Surawska, & Jordan, in review).

At the end of the interview, respondents were instructed on accessing results for the clinically relevant vaginal swab assays (presence or absence of BV, VC, or HPV). Respondents were given an identification number and asked to create a unique four-digit password to access test results. At 3 and 5 weeks following the interview, willing respondents received a reminder letter indicating availability of anonymous results counseling and reporting at ASHA. Via a toll-free phone call, ASHA provided respondents with BV, VC, and HPV test results, pre- and posttest counseling, supplementary educational literature, and referrals. To test the feasibility of a passive survey for results counseling, ASHA counselors completed a postcall survey for a subset of calls, documenting the counseling topics discussed, who initiated the counseling, and about whom the caller was concerned. Callers were anonymous; these data are therefore not linkable to individual-level data in the main NSHAP data set but are in the process of becoming available as part of the NSHAP data set (http://www.icpsr.umich.edu/NACDA/).

Transportation and tracking protocol.—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 Research Institute laboratory, 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. Weekly reconciliation between NORC and the Magee-Women's laboratory compared questionnaire data (indicating whether a sample was recorded as collected) with laboratory data (indicating whether a sample was recorded as received). Discrepancies in reconciliation were resolved and recorded.

Vaginal Self-Swab Assays

The technical details of the vaginal swab laboratory assays have been previously described (Lindau et al., 2008a, 2008b, 2008c; Lindau et al., in review) and are summarized in Table 2.

The availability of specimens for a given assay depended on: (a) participants providing a vaginal swab specimen, (b) the success of the field protocol in delivering the swabs to the laboratories, and (c) the adequacy of the swab for each of the different assays. The specimen adequacy rate for each assay is defined as the ratio of vaginal swab specimens that were eligible for analysis by the laboratory to the total number of specimens obtained in the home eligible for that assay (excluding one specimen lost in the mail).

BV assay and scoring procedure.—Preparation for the BV and VC assays began with rolling of the culture swab onto the surface of a glass slide, thereby transferring the vaginal cellular specimen from the swab to the slide. The slide was air-dried and fixed with 95% methanol and then stained using a standard Gram stain protocol (Burke, 1922).

For BV assessment, 20 or more fields of the stained slide were examined microscopically using an oil immersion objective (×1,000) (i.e., 20 high-power fields). The relative number of each of the three classes of observed vaginal bacterial organisms including (a) *Lactobacillus* species, (b) *Gardnerella* species and anaerobic gram-negative rods, and (c) *Mobiluncus* species were observed. A 0- to 10-point scoring system (previously described [Lindau et al., 2008a]) clinically classifies the specimen as normal vaginal flora/no BV detected (0–3 points), intermediate (4–6 points), and indicative of BV (7–10 points) (Nugent, Krohn, & Hillier, 1991) (Table 3). Slides lacking normal flora (estrogen-related depletion of lactobacilli can be a nonpathologic state in postmenopausal women) and lacking criteria for BV were separately coded. Estrogen-related shifts in the presence of lactobacilli in postmenopausal women may affect interpretation of the Nugent classification; this should be considered in interpreting these scores (Cauci et al., 2002).

VC assay and scoring procedure.—The same gram-stained slides used to assess BV were also evaluated for the presence of yeast (for additional details, see Lindau et al., 2008b). The reader examined the entire area of each stained slide to determine the presence or absence of yeast cells showing blastoconidia (cell buds) in the specimen. A dichotomous score was assigned to each slide. Figure 2 provides photographs of a normal vaginal epithelial cell, bacterial vaginosis (BV) and vaginal candidiasis (VC).

Genital human papillomavirus assays and scoring procedures.—The details of the HPV assays have been previously published, including a flowchart summarizing the disposition of the assays (Lindau et al., 2008c; Lindau et al., in review).

Table 2. Summary of Vaginal Swab Assays Obtained From Self-Swab Specimens in NSHAP

	Collection Device	Manufacturer	Laboratory	Assay Type	Protocol	Coding
BV	Double Copan swab with Dacron tip	BBL™ CultureSwab™ Plus, Catalog No. 220117; Becton, Dickinson and Company	UP-MWRI	Gram stain of vaginal material on glass slide (Burke, 1922)	Clinical	Nugent et al. (1991) scoring system
VC	Double Copan swab with Dacron tip	BBL™ CultureSwab™ Plus, Catalog No. 220117; Becton, Dickinson and Company	UP-MWRI	Gram stain of vaginal material on glass slide (Burke, 1922)	Clinical	Presence or absence of fungal organisms (Joesoef, Hillier, et al., 1991)
HPV						
HR-HPV DNA detection	Female swab specimen collection kit	Catalog No. 5123-1220; Digene Corporation	UP-MWRI	Hybrid Capture 2® (hc2) High-Risk HPV DNA Test™, Catalog No. 5199–1220; Digene Corporation	Clinical	Presence or absence of HR-HPV (13 subtypes)
Genotyping	Female swab specimen collection kit	Catalog No. 5123-1220; Digene Corporation	UP-MWRI	Linear Array HPV Genotyping Test and Linear Array Detection Kit (Roche Molecular Systems Inc.)	Experimental (not FDA approved for clinical use)	Presence or absence of each of 47 anogenital genotypes
Vaginal cytology	Double Copan swab with Dacron tip	BBL™ CultureSwab™ Plus, Catalog No. 220117; Becton, Dickinson and Company	UCIMB University of Chicago Medical Center Department of Pathology	Papanicolaou stain of eluted vaginal material on glass slide, University of Chicago Department of Pathology	Experimental (not conducted in CLIA-approved laboratory)	Vaginal maturation index, cellular density (Bibbo, 1997)

Note: NSHAP = National Social Life, Health, and Aging Project; VC = vaginal candidiasis; BV = bacterial vaginosis; HPV, human papillomavirus; HR-HPV, high-risk human papillomavirus; CLIA = Clinical Laboratory Improvement Amendments; UP-MWRI = University of Pittsburgh, Magee-Women's Research Institute; UCIMB = University of Chicago Institute of the Mind and Biology; FDA = US Food and Drug Administration.

Table 3. Nugent's Point Scoring System for Bacterial Vaginosis

Number of Organisms/×1,000 Objective	0	<1	1–4	5–30	>30
Morphotype					
Lactobacillus species	4	3	2	1	0
Gardnerella species and anaerobic GNR	0	1	2	3	4
Mobiluncus species	0	1	1	2	2

Note: Adapted from Nugent et al. (1991). GNR = gram-negative rods.

HR-HPV detection by hc2 assay.—HR-HPV DNA testing was performed on 1,010 specimens using the Hybrid Capture 2® (hc2) High-Risk HPV DNA Test™ (Catalog No. 5199–1220; Digene, Gaithersburg, MD) according to the manufacturer's protocol. Specimens with relative light unit/cutoff values ≥1.0, obtained using the Digene Microplate Luminometer 2000 (DML 2000™) Instrument, were considered positive for HR-HPV DNA.

HPV genotyping.—Genotyping was performed on 56 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. Due to accessibility of the genotyping assay to our research team in relation to the study period, only hc2 HR-HPV–positive specimens were genotyped.

Coding.—HPV-related variables in the NSHAP data set are not included in the public-use data set, as explained in the *Human Subjects* section, above. Each specimen was reported as positive or negative by hc2 and coded dichotomously. For those testing positive for HR-HPV by hc2, a dichotomous variable based on the Roche genotyping assay result indicates the presence or absence of each of 37 genotypes.

Vaginal cytology assessment and scoring.—Preparation of the cytology slides.—The vials containing BBL™ CultureSwab™ Plus Amies Medium Without Charcoal BD (Becton, Dickinson and Company) were delivered in batches packaged with cold packs from the Jordan Magee-Women's laboratory and were immediately stored at 4°C in a cold room. The vaginal swabs were kept in their individual vials until the sample of vaginal epithelium was transferred from swab to slide. Throughout the transfer process, the vials were placed on wet ice and processed one at a time.

Each swab was placed into a prelabeled 1.5-ml microcentrifuge tube (Catalog No. 05-406-16; FisherF Scientific, Pittsburgh, PA) filled to 1.0 ml with Hanks balanced salt solution (Catalog No. H6648; Sigma, St. Louis, MO). The solution was used to suspend the vaginal epithelial cells from the swab, as well as to thin the Amies medium to facilitate suspension. The swab was gently twirled in the solution for 15 s and discarded. The culture tube, containing the remaining cells in the Amies medium, was recapped, placed on ice, and returned to storage at 4°C along with the microcentrifuge tube containing the suspended cells.

The vaginal epithelial cells typically settled in the tip of the microcentrifuge tube and were resuspended by gentle agitation by flicking the tube with an index finger (mechanical methods degraded the cell suspension). Five slides with triplicate samples were created to maximize validity, reliability, and versatility. First, a 0.15-µl aliquot of the suspended-cell Hanks solution was micropipetted onto the frosted end of the microscope slide (25 × 75 mm) (Catalog No.154003; Propper, VWR, West Chester, PA). Two additional aliquots were placed on the same slide; the size of the aliquot and glass texture ensured that the aliquots remained separate. Five slides from each woman's specimen were prepared, yielding 15 independent samples of vaginal epithelial cells.

Two of the slides (six aliquots), designated for cytologic analysis, were immediately sprayed with an alcohol fixative (Catalog No. 14-372-32; Safetex, Fisher Scientific) and left to air-dry along with the three additional, unfixed slides. After drying overnight, one cytology slide was stained by the University of Chicago Hospitals Department of Surgical Pathology with the progressive Papanicolaou stain (Pap stain) (Bibbo, 1997) using the 24-station Shandon Varistain XY machine. The other slide, fixed for cytology staining, was stored in microscope boxes at room temperature for future use. The unstained slides were stored in microscope slide boxes in a 4 °C cold room for future analyses.

Pap staining facilitates cytological coding by adding color that helps distinguish superficial epithelial cell types from those deriving from deeper layers of the vaginal epithelium and differentiates nuclear from cytoplasmic material. Estrogen promotes cellular glycogen production (the substrate for lactobacilli, a predominant bacteria normally residing in the vagina that is necessary for maintaining acidity [pH]). Typically, glycogen in the estrogenized superficial cells of the vaginal epithelium stains red, revealing a pyknotic nucleus (Papanicolaou, 1942, 1956). Cells from the intermediate epithelial cell layer stain light blue and have a less dense nucleus compared with superficial cells (Papanicolaou, 1956). Cells from the parabasal layer stain dark blue and have a larger nucleus than the intermediate cells. Basal cells, from the deepest epithelial layer, stain dark purple and have the largest nucleus of all the cell types above (Papanicolaou, 1956). These four cell types are also distinguished from one another by size, the nuclear to cytoplasm ratio, and the characteristics of their nuclei. Illustrative photographs are presented in the *Results* section.

The vaginal maturation index.—The vaginal maturation index (MI), a common clinical measure, uses the exfoliated vaginal cells to evaluate the hormonal status and thickness of the vaginal epithelium (Cibas & Ducatman, 2003). The MI also gives insight into the estrogen status of the related genital structures including the vulva, urethra, and the bladder (Grodstein, Lifford, Resnick, & Curhan, 2004;

Hammond, 1977). In contrast to a serum or salivary measure of circulating estrogen, the MI indicates the net effect of biologically active sex hormones (circulating levels of free estrogens, androgens, and progestogens) on the vaginal epithelium and provides an integrated measure of hormonal bioactivity over time, rather than blood levels at a single time point (McEndree, 1999). The traditional MI quantifies the relative proportion of the vaginal parabasal (P), intermediate (I), and superficial (S) cells (Bibbo, 1997).

Coders from the Institute for Mind and Biology, experienced in cytology assessment of the rat vaginal epithelium, were trained by clinical cytopathologists at the University of Chicago Hospitals to read slides for the MI; coders were not trained to recognize cellular atypia. First, using a modification of the Cibas and Ducatman method, a visual survey approach at ×100 magnification was used to read each slide systematically from left to right, checking each aliquot for cellular number, quality, and consistency (Cibas & Ducatman, 2003). Slides with at least 100 densely stained cells, consistent in proportions across the three aliquots, were considered "excellent" for coding the MI. Slides were deemed "adequate" if they had one of the following characteristics: moderate staining, fewer than 100 cells, unstained nuclei, or cell fragments (see photographs in *Results*). Slides with several of these characteristics were deemed inadequate for traditional MI methodology. The coder then estimated the relative proportion of parabasal, intermediate, and superficial cells (P:I:S), integrating across all three aliquots.

Specimens were obtained for assessment of vaginal, not cervical, cytological characteristics. Cervical cells are needed in order to assess risk for cervical cancer (e.g., Papanicolaou smear). Self-collection of cervical epithelial specimens for cytopathology requires a different approach, including clinical laboratory assessment and clinical-level results counseling; this was outside the scope of our study.

Results

Participant Cooperation in Vaginal Self-Sampling

Of the 1,550 women, 1,028 (66.3% unweighted, 67.5% weighted) agreed to submit a self-administered vaginal swab specimen (Figure 3). A total of 522 women were considered nonresponders; 521 respondents did not submit a swab specimen, and for one individual the vaginal swab protocol was inadvertently omitted due to a data entry error earlier in the interview.

In univariate analysis, participants in the vaginal swab collection were significantly more likely to be younger (<74 years), and have at least a high school diploma, and were more likely to report a recent pelvic examination, menopausal prescription hormone use, and sexual activity in the past year (Table 4). All female participants were interviewed by a female field interviewer. Field staff whose age matched that of the study population were significantly more likely to obtain cooperation with the vaginal self-swab protocol (74.5% of those 57–85 years old vs. 62.4% of those 28–56 years old, $p < .001$). Field staff were encouraged to record the respondent's reason for refusal, if offered. When recorded, the most common anecdotal reason for nonparticipation was a physical or health problem.

Specimen Adequacy

The vast majority of the vaginal self-swab specimens obtained in the home arrived in the laboratories and were adequate to be processed for the assays: 957/1,016 (94.2%) for BV and VC, 1,010/1,012 (99.8%) for human papillomavirus (HPV hc2) testing, and 1,016/1,016 (100%) for cytology. For BV and VC, of the 1,016 samples processed, 58 (5.7%) had an insufficient quantity of bacterial or fungal material and so were inadequate for analysis; 51(5.0%) were adequate for analysis but not assigned a Nugent score because they lacked both normal and BV-like vaginal flora. Details regarding the HPV specimens have been previously published (Lindau et al., in review); however, among the 1,010 adequate for analysis, all were adequate for testing by the hc2 assay, and 56 of 64 (87.5%) specimens testing positive for HR-HPV by hc2 were adequate for genotyping (Figure 3).

All 1,016 swabs received by the Jordan Laboratory were sent on to the McClintock Laboratory, and all were processed for cytological analysis. Of these, 85.5% were excellent or adequate for traditional MI analysis (869/1,016; Figure 3). As shown in Figure 4, all three types of vaginal epithelial cells—superficial, intermediate, and parabasal—used in the MI were readily identified in the adequately stained slides. The majority of the Pap-stained cytology slides that were categorized for MI had more than 100 cells (722/869, 83%; see Figure 5).

There was little overlap between specimens found to be inadequate for pathogen analysis (BV, VC) and those found to be inadequate for cytological analysis. Only 0.9% (9/1,016) of the specimens were inadequate for both. In other words, 99.1% of women who provided a swab successfully collected a vaginal specimen.

Results Reporting Usage

All respondents who provided a vaginal swab specimen and/or an HIV test (women and men) were eligible to call ASHA for results counseling and receipt (n = 1,551) and were provided detailed instructions at the time of the interview about how to access results. Of the respondents eligible to call ASHA, 882 (56.9%) also agreed to receive mail notification of results availability. A total of 685 (44.2%) calls were made to the NSHAP results hotline. The content of 517 (75.5%) of these counseling conversations were documented by ASHA phone counselors. Due to a small number of repeat callers, ASHA estimated that the true

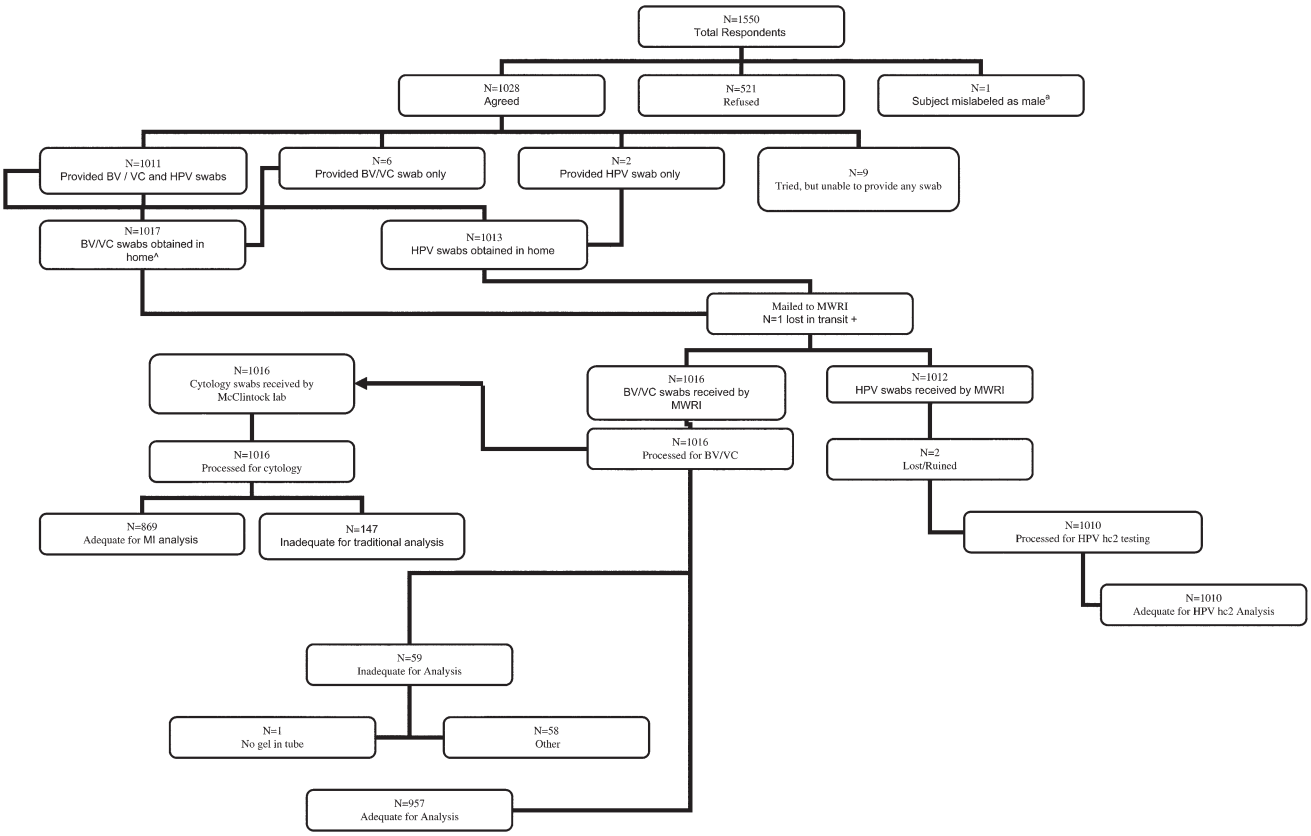

Figure 3. Vaginal self-swab cooperation and specimen disposition[b]. Vaginal swab not offered. These were also used for cytological analysis. +Both the BV/yeast swab and HPV swab from one subject were lost. [b]MWRI, Magee-Women's Research Institute; BV= bacterial vaginosis; VC= vaginal candidiasis; HPV= human papillomavirus; HPV hc2= Hybrid Capture 2® High-Risk HPV DNA Test™.

percentage of respondents seeking results was closer to 41%–42% of those eligible for results. Although data on counseling about BV and VC were collected, they are very limited due to a high frequency of missing data (resulting from a programming error in the ASHA electronic passive survey) and are therefore not reported here. However, HPV counseling data were successfully obtained.

Among the 517 documented calls, HR-HPV results were provided to 335 callers, and 100 of these individuals (29.9%) had questions about HPV. Counseling included definition of the disease, test accuracy, symptoms, transmission, risk reduction, treatment, and emotional issues. Respondents rarely initiated discussions. The most frequently discussed topics included definition of the disease and symptoms; emotional issues were the least frequently discussed. For each counseling topic discussed, the counselor identified about whom the respondent was concerned—self, partner, family, or other. Most callers did not identify a specific individual of concern, but for those who did, they most often expressed concern about self rather than others.

Discussion

The acceptability and performance of vaginal self-swab specimens in studies of younger women are well established and compare very favorably to specimens collected by clinical transvaginal speculum examination (Chernesky et al., 2005). Interdisciplinary collaboration among NSHAP investigators merged clinical medical with social survey and behavioral endocrinology expertise to successfully implement novel methods for vaginal self-swab specimen collection with older community-residing women participants and nonmedical field staff. Self-swab participation rates overall (number of collected swabs/number of eligible study participants) were excellent, particularly given prevalent functional limitations such as arthritis and obesity that could interfere with older women's physical ability to accomplish specimen collection (Drum, Shiovitz, Gaumer, Surawska, & Lindau, in review).

Although the participation rate was lower in NSHAP (66.3%) than in a recent NHANES study of HPV in younger women (ages 18–59 years) using similar self-swab methods (81.6%) (Dunne et al., 2007), the specimen adequacy rate for HR-HPV (number of adequate swabs/number of collected swabs) exceeded that reported by NHANES (Lindau et al., in review), and NSHAP specimen adequacy rates for BV, VC, and cytology were also very high. In contrast to NSHAP, NHANES' self-swab specimens of younger women were obtained in a mobile clinical setting, with medical personnel, and using specimen storage and laboratory transport

Table 4. Bivariate Correlates of Participation in NSHAP Vaginal Self-Swab Protocol

	Participants (%)	Nonparticipants (%)
Participant demographic characteristics		
Age (years)***		
57–64	41.0	35.4
65–74	36.4	31.4
75–85	22.6	33.3
Race or ethnic group		
White	80.4	80.9
Black	10.6	11.3
Hispanic/non-Black	7.10	5.70
Other	1.90	2.10
Marital status		
Married	56.4	53.5
Living with a partner	2.50	2.10
Separated	1.30	0.60
Divorced	13.7	11.2
Widowed	23.5	28.3
Never married	3.00	4.30
Educational status*		
<High school graduate	18.3	25.5
High school graduate	31.5	30.2
Some college or associate's degree	31.8	25.7
College graduate	18.4	18.6
Insurance status		
Medicare	63.5	64.7
Private without medicare	32.1	29.6
VA, other, Medicaid	3.70	4.00
None	0.72	1.60
Participant health characteristics		
Self-rated health		
Poor	5.69	8.25
Fair	17.5	18.1
Good	31.3	32.0
Very good	32.8	28.3
Excellent	12.8	13.3
# Comorbidities		
0–1	54.3	58.6
2	25.0	20.9
3–9	20.7	20.5
Diagnosed with cervical dysplasia*		
Yes	9.95	8.96
No	90.1	91.0
Smoking status		
Nonsmoker	50.2	49.6
Past smoker	35.6	36.2
Current smoker	14.2	14.2
Hysterectomy		
Yes	45.6	41.4
No	54.4	58.6
Sex in the last year*		
Yes	45.0	37.3
No	55.0	62.7
Time since last Pap smear*		
Within the past year	44.3	42.8
1–5 years	37.5	31.5
>5 years	16.7	23.1
Never	1.50	2.70
Postmenopause hormone use**		
Yes	53.2	42.9
No	46.8	57.1

Notes: NSHAP = National Social Life, Health, and Aging Project.
*$p < .05$, **$p < .01$, ***$p < .001$.

procedures typical of clinical protocols. The similarity in performance of the vaginal self-swab methods across these two major population studies, despite the differences in study population and research setting, lends additional reassurance about the feasibility of the NSHAP vaginal self-swab methods for use with population-based samples of older women.

Collection of vaginal cytological data in the population setting, particularly from older women, was both highly novel and highly successful. Interpretation of the MI for managing gynecologic problems in the clinical setting has been based heavily on small, referent data sets from individual physicians' clinical practices and is therefore subject to selection bias (Hafez & Evans, 1978). The Pap-stained cytological specimens obtained in NSHAP are indistinguishable from those obtained under clinical conditions. Although no data are available for comparison, clinical experience suggests that the specimen adequacy rate is also comparable. The NSHAP vaginal self-swab protocol for cytological assessment presents a highly promising, new field-based biomeasure for use in population-based older women's health research.

Several factors emerge in bivariate analysis to explain cooperation with the vaginal self-swab protocol, including respondent sociodemographic and health characteristics and field staff characteristics. In contrast to studies of younger women where sociodemographic factors are not significantly associated with self-swab participation (Chernesky et al., 2005), we found that younger, more highly educated, healthier, sexually active respondents and those indicating participation in gynecologic care were more likely to participate. Interestingly, in bivariate analysis, field staff whose age matched that of the study population were significantly more likely to obtain cooperation with the vaginal self-swab protocol. Concordance between respondent and interviewer sociodemographic and/or racial characteristics has been found to be influential in some cases (Campanelli & O'Muircheartaigh, 1999; Pickery & Loosveldt, 2002), and accounting for such interactions could be informative. Other mechanisms through which field staff characteristics may influence respondent cooperation include field staff experience and confidence (Groves & Couper, 1998). For future waves of NSHAP, the impact of both general interviewing experience and prior NSHAP interviewing experience on participation could be explored.

During the field period, booster trainings were implemented for a subset of interviewers to maximize respondent cooperation; the effectiveness of this intervention may be reflected by stable vaginal swab cooperation rates throughout the study period. Alternately, without additional training during the data collection period, one might have expected cooperation with vaginal self-swab collection to be subject to decline over time as the more reluctant or inaccessible participants were recruited into the study. Of course, some cases of missing vaginal swab data are due neither to

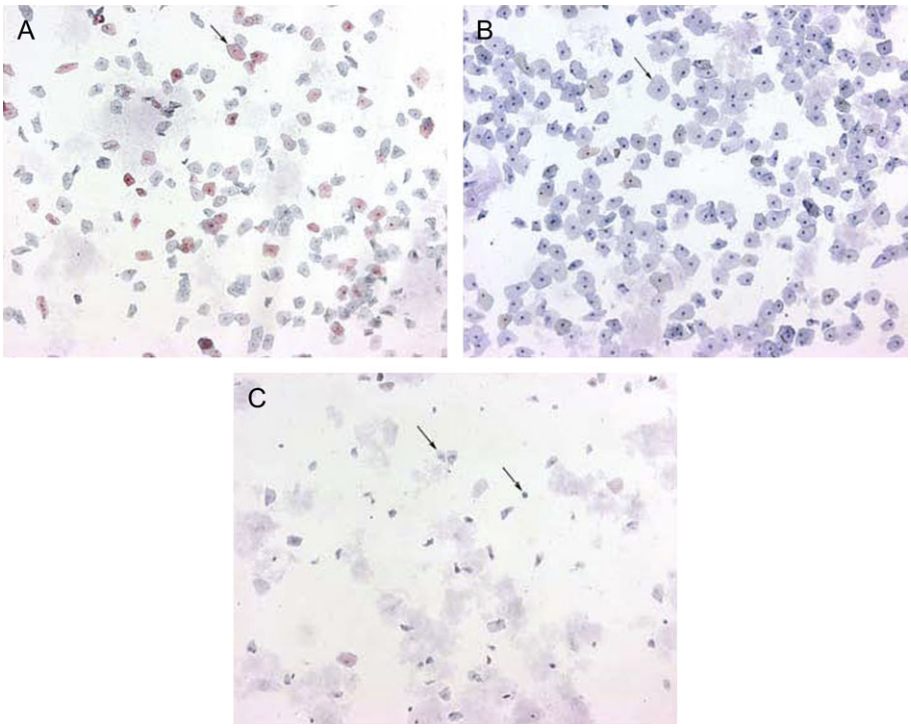

Figure 4. Variations of the vaginal maturation index (MI) from NSHAP self-swab specimens. (**A**) An estrogenized sample of superficial cells and intermediate cells; MI = 0:60:40, Pap stain, ×100, arrow designates a superficial cell. (**B**) A moderately estrogenized sample of intermediate cells; MI = 0:100:0, Pap stain, ×100, arrow designates an intermediate cell. (**C**) An atrophic sample of parabasal, intermediate, and superficial cells; MI = 70:30:0, Pap stain, ×100, upper arrow designates a parabasal cell (lower arrow designates a basal cell). All images were captured with Carl Zeiss Axioskop microscope and Carl Zeiss Axiocam Color camera. Image acquired using Improvision Openlab Imaging Software.

the respondent nor to the field staff person but to errors in equipment, transportation, and laboratory processing (as indicated in Figure 3). Interventions to address these sources of missing data would need to occur in conjunction with strategies targeted to respondents or field staff. Further analyses of field staff correlates of participation could be accomplished with access to NSHAP operations data; however, these are currently not part of the NSHAP public use data set.

An anonymous results reporting system provided counseling and results to the respondents who chose to call for their vaginal swab test results. Most importantly, all callers seeking results were able to receive their results. This demonstrates the operational success of the anonymous results reporting system, which required flawless communication among home, Magee-Women's laboratory, NORC, and ASHA.

All respondents providing specimens for which counseling was available were given instructions during the in-home interview for test results access, but more than 40% declined further notification of results availability, and a minority of those eligible actually called the ASHA hotline. Studies of younger adults also find that many respondents decline to access test results following study participation (Pugatch et al., 2001). This may be due to active avoidance of the information, disinterest, or the feeling that the information replicates testing already received in the clinical setting. For older adults, difficulty with phone communication due to hearing or speech problems may also present a barrier. In the case of NSHAP, counseling data collected by the pilot passive survey were limited by a technical error in survey programming by ASHA that resulted in inadvertent skipping of questions relating to BV and VC. This can easily be overcome for future waves. Although the cooperation data are limited, they do shed light on the role and value of a results counseling service for vaginal swab–based testing in a population-based study that includes older women and the feasibility of implementing a passive survey in conjunction with the phone encounters. Interpretation of these data would be significantly enhanced if linkable to the main data set; how to accomplish this while protecting participant anonymity presents a challenge that we were not able to overcome in the NSHAP study.

Experience from the NSHAP study demonstrates that collection of vaginal self-swab specimens from older women in a population-based in-home study using nonmedical field staff is feasible. The quality of the self-swab specimens is high, as indicated by a high specimen adequacy rate for all of the assays. Vaginal swab specimens provide novel data on microenvironmental characteristics of the female genital tract relevant to analyses of gynecologic health and sex hormone metabolism, sexual activity

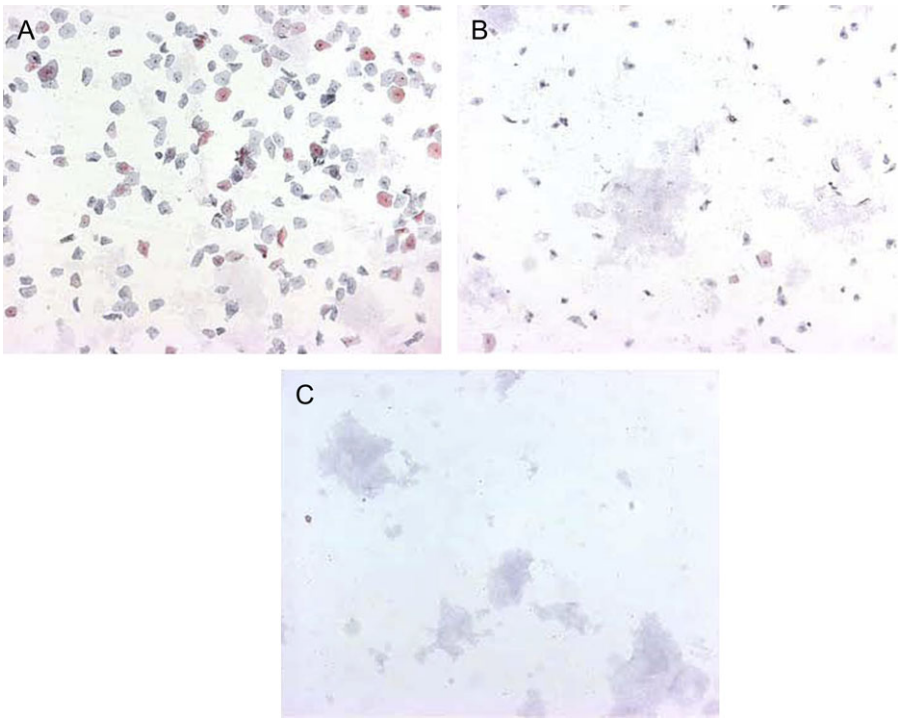

Figure 5. Vaginal epithelial samples from NSHAP self-swab specimens categorized as excellent, (**A**) adequate, and inadequate based on the cell number criterion. (**B**) Adequate sample, 50–99 cells visible, Pap stain, ×100. (**C**) Inadequate sample, fewer than 50 cells visible, Pap stain, ×100. All images were captured with Carl Zeiss Axioskop microscope and Carl Zeiss Axiocam Color camera. Images acquired using Improvision Openlab Imaging Software.

and problems, and the immune and inflammatory status of older women.

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