Bacterial Vaginosis

Vaginal Swab Measurement of Bacterial Vaginosis in Wave I of the National Social Life Health & Aging Project

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Rationale

Bacterial Vaginosis (BV) is a common polymicrobial condition of the vagina that occurs with reduction of vaginal acidity (pH) and a shift in the normal bacterial flora. In the younger population, BV is associated with douching (LlahiCamp, Rai et al. 1996), sexual intercourse with multiple partners, oral sex with different sexual partners, and cigarette smoking (Smart, Singal et al. 2004). Furthermore, BV in younger

women has been associated with infection in the lining of the uterus and upper genital tract, which in turn, can elevate the risk of infectious complications following the gynecologic disorder (Lindau, Hoffmann et al. Under Review). BV also increases the risk of sexually transmitted infections such as HIV (Sewankambo 1997; Sobel 2007). While BV is common among women of reproductive-age, information is limited regarding BV prevalence in older women (Allsworth and Peipert 2007).

Measurement

Clinical diagnosis of BV can be characterized by the following criteria: 1) thin, off-white vaginal discharge with a "fishy" odor documented by amine testing 2), abnormal cervical discharge and vaginal bleeding (especially after intercourse) 3) a pH in excess of 4.7, and 4) clue cells on microscopic evaluation of saline wet preparation(Sobel 2007 Lancet). These criteria are based on Amsel's work, and are still used today (Amsel, Totten et al. 1983; Gutman, Peipert et al. 2005). Alternatively, Gram staining is performed on a vaginal smear and scored using either the Hay/Ison (Ison and Hay 2002) or Nugent criteria (Nugent, Krohn et al. 1991).

Population Prevalence

Bacterial Vaginosis is a common vaginal disorder among women (Sobel 2005). It accounts for up to 50% of cases of vaginal discharge in non-pregnant women. (Sobel, Ferris et al. 2006). However, BV may be asymptomatic (Sobel 2007 Lancet) or variably symptomatic (Klebanoff, Schwebke et al. 2004).

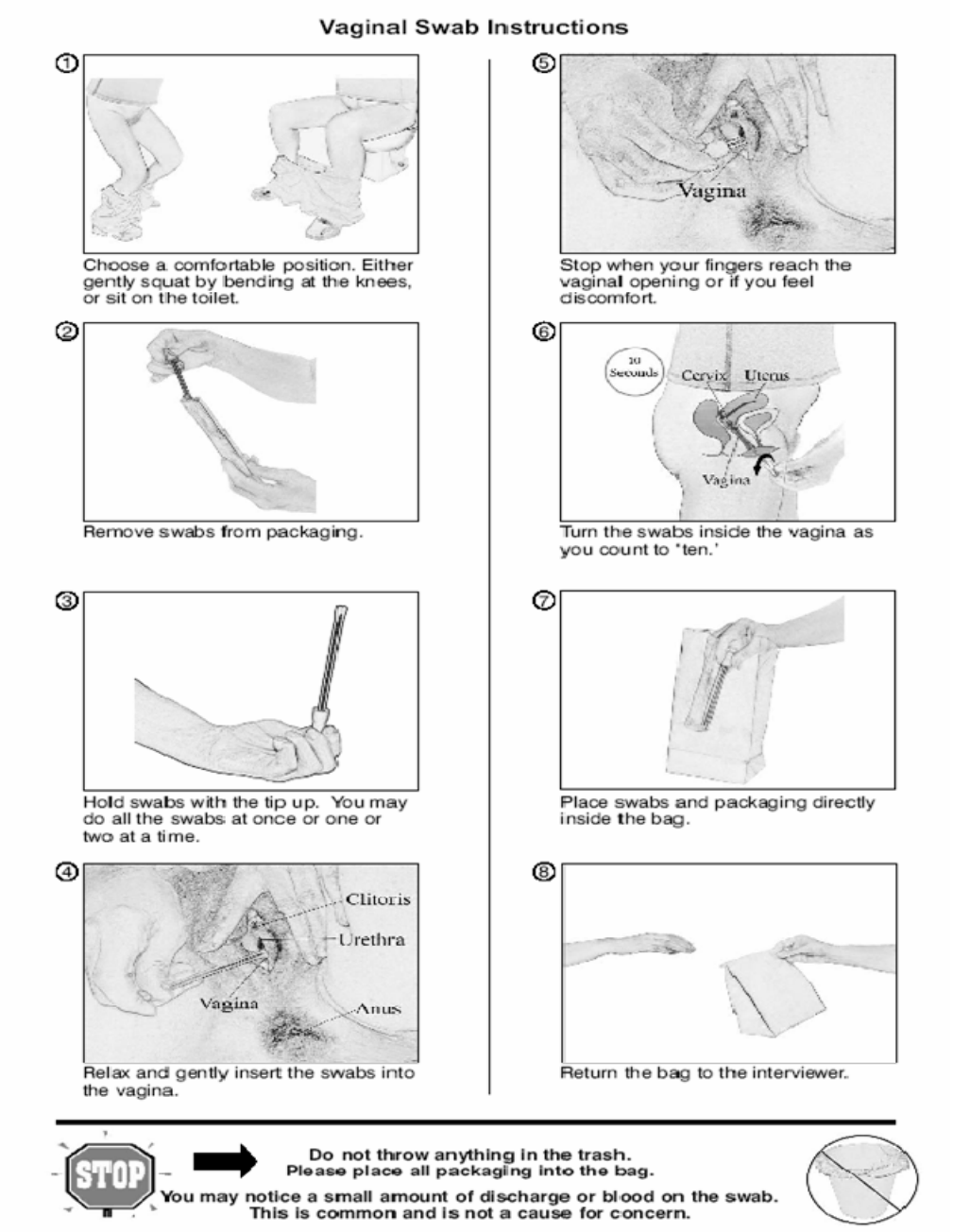
In the data from the National Health and Nutrition Examination Survey, BV prevalence was estimated to be 29% among the general population of women ages 14-49, using a self-swab protocol. The prevalence among younger women (14-19 years) was lower (23.3%) than among women 40-49 years (31.3%) (Allsworth and Peipert 2007). Data regarding BV prevalence in post-menopausal women are limited (Cauci, Driussi et al. 2002). In a northern Italian study among non-pregnant women (40-79 years) 6% of postmenopausal women, 11% of perimenopausal women, and 9.8 % of fertile women met microbiologic criteria for a diagnosis of BV (Cauci, Driussi et al. 2002).

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%(Lindau, Hoffmann et al. Under Review).

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.

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. Specimens were shipped daily.

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

A Gram stained slide of a smear made from the vaginal swab samples was made in the Magee-Women's Research Institute laboratory of JAJ. The smear was evaluated for bacterial morphotypes and interpreted in accordance with pre-existing criteria. Nugent's criteria (Nugent, Krohn et al. 1991) for a standardized 0-10 point scoring system were used to interpret the Gram stain result. Zero to 3 was classified as normal/no BV detected, 4-6 as intermediate, and 7-10 indicated the presence of BV. Each stained slide was read by two independent observers who were blinded to the other's results. Discordant results were re-reviewed by the initial two readers as well as reviewed by a third observer to resolve the discrepancies.

Table 1. NSHAP Vaginal Swab testing performed by Magee-Women's Research Institute

Assay Type	*Assay principle*	*Regulatory Status*	*Samples*
Gram stain smear	Evaluation of bacterial morphotypes	None	Vaginal or cervical self-collected swab

The same gram stained slides used to assess the presence of bacterial vaginosis were also evaluated for the presence of yeast, by using the 1000x objective. A dichotomous score was assigned to each specimen slide, after the reader viewed and examined the entire area of each stained slide to determine the presence or absence of yeast cells showing blastoconidia (cell buds)(Lindau, Hoffmann et al. Under Review).

Technical details of the vaginal swab laboratory assays have been previously described (Lindau, Drum et al. Under Review). The BV assay technique is summarized in the following table

Table 2. Summary of Vaginal Swab Assays Obtained from Self-Swab Specimens in NSHAP[†]

	Collection Device	Manufacturer	Laboratory	Assay Type	Protocol
Bacterial Vaginosis (BV)	Double Copan swab with Dacron tip	BBL ™ CultureSwab™ Plus, Catalog No. 220117; Becton,	UP-MWRI	Gram stain of vaginal material on glass slide (Burke, 1922)	Clinical

		Dickinson and Company, Franklin Lakes, NJ			

Quality Control

The laboratory participates in semi-annual proficiency testing for gram staining and BV interpretation, and must achieve greater than 90% overall agreement with these challenges. Gram stain reagents are checked weekly and with each new lot of stain before they are put into use. Gram stain reagents were evaluated by staining ATCC 25923 Staphylococcus aureus, ATCC 25922 E. coli, and ATCC 26555 Candida albicans.

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, insert the swab into the STM tube and break off the extra handle of the swab by pressing it along the side of the tube. Labelthe tube with 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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