Variability of Vaginal pH Determination by Patients and Clinicians

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Purpose: **Measurement of intravaginal pH provides a reasonable assessment of vaginal health but is fraught with the potential for several sampling errors. The purposes of the study were to determine the variability of self-sampled vaginal pH among women using an inexpensive swab-based pH test compared with a clinician-obtained specimen, and variability of vaginal pH within 3 regions of the normal vagina.**

Methods: **In this cross-sectional study, women obtained a vaginal specimen using a cotton-tip applicator, transferred it to pH paper, and interpreted the results. A clinician also blindly interpreted these tests. Thereafter, a clinician obtained 3 swab specimens from the proximal, middle, and distal vagina for pH testing. Results were compared using Wilcoxon signed rank test, interclass correlation coefficients, Bland-Altman plots, and mixed-model analysis of variance.**

Results: **Interclass correlation coefficients were moderately high comparing subjects with clinician for the swab-based pH test (0.74). Subjects' swab pH values (4.5) were significantly lower than clinicians' pH values (4.7, *P* = .0001). Intravaginal pH did not vary between the 3 anatomic locations.**

Conclusions: **Self-sampled intravaginal pH interpretations vary slightly compared with clinician-obtained specimens. Because swab pH sampling does not detect an intravaginal pH gradient in normal women, self-sampling technique may vary considerably without affecting outcomes. Our findings support self-sampling for vaginal pH before using over-the-counter products for presumed vaginitis. (J Am Board Fam Med 2006;19:368–73.)**

The normal vaginal ecosystem in mature women is maintained by Lactobacilli species that secrete lactic acid and hydrogen peroxide. A resulting pH <4.7 is thought to limit the overgrowth of opportunistic microbes. Consequently, vaginal secretions are clear, non-irritating and void of offensive odor. More importantly, potentially serious gynecological and obstetrical complications arising from an abnormal vaginal ecosystem associated with bacterial vaginosis are prevented.[1]

Assessment of intravaginal pH is a helpful, but frequently neglected, diagnostic procedure used to evaluate vaginal health.[2] Because microscopic eval-

uation of vaginal secretions is prone to misdiagnosis, a simple pH determination assumes even greater importance with respect to Amsel's criteria used to clinically diagnose bacterial vaginosis. Clinicians usually obtain vaginal secretions or discharge from the lateral vaginal walls with a small cotton-tip applicator. The moist specimen is then transferred to a strip of pH paper and compared with a standardized colorimetric reference chart to estimate actual pH. With respect to diagnosing vaginitis, an elevated pH suggests Trichomonas vaginitis or bacterial vaginosis. Vulvovaginal candidiasis is generally detected in a normal vaginal pH range (<4.7).

Patient self-diagnosis and self-treatment of vulvovaginal candidiasis is fraught with error.[3–6] Two-thirds of women who purchase over-the-counter antimycotics for treatment of self-diagnosed vulvovaginal candidiasis do not have vulvovaginal candidiasis.[3] An easy, rapid and inexpensive self-diagnostic test for vaginitis may help to minimize the tendency to self-treat vaginitis inappropriately. Self-collected vaginal samples may be as accurate as clinician-obtained specimens for diagnosing various sexually transmitted infections including *Neis-*

seria gonorrhea, *Chlamydia trachomatis*, *Trichomonas vaginalis*, and human papillomavirus.[7–13] Moreover, self-sampling the vagina seems to be very acceptable to women of multiple ethnic groups.[8,9,13–17] Women have also demonstrated a willingness to obtain a self-sample of vaginal secretions for Gram stain detection of bacterial vaginosis.[18] A self pH test of vaginal secretions would permit a relatively simpler appraisal of vaginal ecosystem status.[19,20] However, pH within the vagina is not uniform and depending on location, becomes less acidic toward the introitus.[21] In addition, false elevations of pH may be encountered when semen, mucus, or blood is inadvertently sampled. Because self-sampling of vaginal secretions may vary in technique (depth, duration, and position) among women, pH results may also vary accordingly. The purpose of this study was to determine the variability of self-sampled vaginal pH among normal women using an inexpensive swab-based pH assessment method compared with clinician-obtained specimens. We also assessed variability of vaginal pH within 3 regions of the vagina as measured by a clinician. The ultimate goal of our research program is to enhance knowledge and proper use of vaginal pH self-sampling, particularly for women considering over the counter treatment of presumed vulvovaginal candidiasis.

Subjects and Methods

A convenience sample of women 18 years of age and older, and without vaginal symptoms, was passively recruited (using pamphlets) to participate from clinics at the Medical College of Georgia. Women who were pregnant, menstruating, or had a known vaginal or cervical infection were excluded. The study was approved by the Institutional Review Board.

All subjects were informed of the cross-sectional study requirements and signed an informed consent document. Only subjects with a negative urine human chorionic gonadotropin test result were allowed to continue. Demographic and gynecologic information was obtained and subjects were asked to read a one-page instruction sheet that described the techniques for self-measurement of vaginal pH. Subjects held a cotton-tip applicator between their thumb and index finger and inserted the swab into the vagina. They then were instructed to rotate the swab clockwise and counter-clockwise while attempting to touch the vaginal sidewalls. Subjects

removed the swab, placed it on a strip of pH paper (pHydrion paper; Micro Essential Laboratory, Brooklyn, NY), and independently compared the color of the pH paper with the standard pH color reference chart (pH 3.0–5.5) to determine their self-obtained pH test results. At the same time, a clinician with normal color vision also independently determined the pH results of subjects' self-obtained specimens. Immediately afterward, the clinician inserted a vaginal speculum and obtained 3 specimens from the proximal, middle, and distal vagina using different cotton-tip applicators. Secretions from the swabs were transferred to other strips of the same type of pH paper (pHydrion) to determine clinician-obtained pH results from the 3 regions of the vagina.

Descriptive statistics were calculated for all demographic and clinical variables. To examine whether the interpretation of pH within the vagina was similar between clinician and subjects, several analyses were performed. First, Wilcoxon's signed rank tests (due to the non-normality of the data) were used to examine whether median pH results were different between subjects and clinician. Second, interclass correlation coefficients (ICC) were determined for reliability of swab-based pH tests for subjects and for clinician. Third, a Bland-Altman plot was constructed for subjects versus clinician for the swab-based pH tests. The intent of this study was to determine concordance and not accuracy of pH determinations because a criterion standard test (in vivo pH meter) was not included. Consequently, the final analysis determined agreement between subject's and clinician normal (<4.7) and abnormal (≥4.7) pH results using κ statistic.

To examine differences in pH level between the proximal, middle, and distal vaginal area, a mixed model analysis was used. First, the unadjusted means were examined in a one-factor model. Next, potential covariates of age, race, parity, hormone replacement therapy, and birth control were added to the one-factor model. Finally, a backward model building process was used to remove any covariate that was not statistically significant at an α level of 0.05. The final model consisted of the vaginal area factor and any covariates that were statistically significant at the 0.05 α level. A Tukey multiple comparison procedure was used to examine pair-wise differences post hoc in the adjusted least square means of the pH in the different vaginal areas.

Table 1. Demographic Statistics (n = 113)

Variable		
Race (n, %)		
Black	53	(46.9)
White	55	(48.7)
Asian	0	(0.0)
Hispanic	3	(2.7)
Other	2	(1.8)
Age (mean, SD)*	35.1	(10.1)
Height (mean, SD)†	64.7	(3.2)
Weight (mean, SD)	171.8	(45.3)
Gravida (mean, SD)	2.0	(1.7)
Parity (mean, SD)	1.7	(1.5)
Abortion (mean, SD)	0.3	(0.7)
Vaginal births (mean, SD)	1.5	(1.5)
Cesarean sections (mean, SD)	0.3	(0.7)
Regular periods (n, %)	78	(69.0)
Pelvic surgery (n, %)	28	(24.8)
Vaginal medications (n, %)	1	(0.9)
Hormone replacement therapy (n, %)	11	(9.7)
Birth control method (n, %)		
None	36	(31.9)
Surgical	27	(23.9)
Other‡	50	(44.3)

* Height in inches.
† Weight in pounds.
‡ Includes birth control pills, barrier methods, progesterone injection, transdermal, intrauterine device, and abstinence.

All statistical significance was assessed using an α level of 0.05, and all statistical analyses were performed using SAS 8.2.

Results

A total of 113 subjects participated in the study. Descriptive statistics for all demographic and clinical variables are given in Table 1. In general, the population could be summarized as representing mainly blacks and whites, middle-aged, overweight, and multiparous women.

Subjects' and clinician mean intravaginal pH interpretations and SD for the subject-collected swab method were 4.5 (0.5) and 4.7 (0.5), respectively.
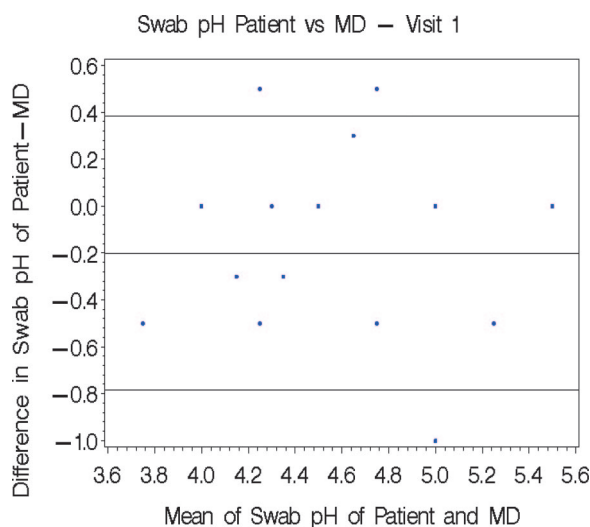

Figure 1. Bland-Altman plot for differences in swab pH as determined by subjects and the clinician. pH values had minimal variability between observers.

ICC and Wilcoxon's signed rank tests were used to compare results between subjects and clinician for the swab-based pH tests (Table 2). Comparing subjects with clinician for the swab-based pH test, the ICC values were moderately high (0.74) indicating good reliability. The Wilcoxon signed rank test result indicated that the median pH values were different. Subjects' pH values (4.5) were significantly lower than clinician pH values (4.6, $P = .0001$). There was moderate agreement between subjects' and clinician interpreted normal and abnormal pH results ($\kappa = 0.67$, 95% CI 0.52–0.81).

A Bland-Altman plot was used to portray variability of pH results using the swab (Figure 1). Most points lie inside the 1.96 SD limits indicating that the agreement between subjects and clinician for swab pH testing was very good.

Mean intravaginal pH results using a clinician-directed swab sampling method of the proximal, middle and distal vagina were calculated. These values varied little; 4.6 (0.7), 4.5 (0.5) and 4.5 (0.4), respectively. A mixed model analysis was then used to determine pH result differences by intravaginal

Table 2. Comparison of Samplers and Intravaginal pH Sampling Methods

Swab-based pH test	Mean pH	SD	Median pH	ICC*	S	P Value
Subject	4.5	0.5	4.5	0.74	−538.0	.0001
Clinician	4.7	0.5	4.6			

* ICC, interclass correlation coefficients; S, Wilcoxon's signed rank test.

Table 3. Mixed Model Analyses for Differences of Intravaginal pH in 3 Regions of the Vagina

| Model | Adjusted Least Square | | F Value | P Value |
	Mean	SE		
Unadjusted model				
Vaginal area			0.84	.43
Proximal	4.60	0.07		
Middle	4.52	0.05		
Distal	4.54	0.04		
Full model				
Age			1.22	.27
Parity			7.04	.01
Race			3.47	.02
Hormone replacement therapy			1.06	.31
Birth control			3.29	.07
Vaginal area			0.61	.54
Proximal	4.59	0.12		
Middle	4.53	0.11		
Distal	4.53	0.11		
Final model				
Parity			10.40	.002
Race			4.17	.008
Vaginal area			0.61	.54
Proximal	4.61	0.11		
Middle	4.55	0.09		
Distal	4.55	0.09		

sampling site (Table 3). There were no differences in pH between the 3 vaginal areas in any models. This indicates that in these women, little variation in the pH was seen regardless of intravaginal sampling area.

Discussion

Experienced clinicians realize the importance of measuring intravaginal pH to determine the status of the vaginal ecosystem. Although ignored by many clinicians, Amsel's criteria for the clinical assessment of bacterial vaginosis considers pH, in addition to amine odor, adherent vaginal discharge, and presence of clue cells.[22] The latter assessment requires a microscope, transport media, glass slides, and cover slips. Moreover, it involves considerable training, careful scrutiny, and several minutes of appraisal. In contrast, the pH test is less complicated, much cheaper and results are available immediately. Because of its simplicity, ability to improve health care, and potential appeal to women, self-assessment of the vagina using pH paper was critically evaluated.

We demonstrated minor differences in pH interpretations between women and a clinician when a swab was used for sampling. Although women reported significantly lower pH values on average when compared with the clinician, these differences were not clinically meaningful. Specifically, the mean pH result by subjects was considered normal, and the clinician's mean pH result may be interpreted as borderline normal. Although moderate agreement was observed between subject's and clinician pH interpretations, it is important to note that the self-testing results of women may vary slightly from their clinicians' results. This potential for minor discordance may occasionally create confusion and management dilemmas for both patients and health care providers. A careful clinical evaluation and further laboratory testing should be undertaken when symptoms or a self pH test result raise diagnostic questions.

Much to our surprise, there was no difference in intravaginal pH by region when assessed by a clinician using a swab and pH paper. Others have described a pH gradient within the normal vagina using more sophisticated testing devices,[21] but the

swab and pH paper method may not have been sensitive enough to detect small changes of pH throughout the normal vagina. In an evaluation of a different pH paper (range 4.5–7.5) compared with a pH meter for assessing intravaginal pH, the pH paper had a correlation coefficient of 0.87, but a mean pH difference of 0.95.[23] Because no in vivo pH meter was used in our study as a criterion standard, we were unable to determine whether women or the clinician rendered more accurate assessments. However, the intent of our study was to determine concordance and not accuracy.

Any variation of pH interpretations in normal asymptomatic women seems to be clinically irrelevant as measured by women and a clinician. Although clinicians can make an effort to avoid sampling mucus, blood, and semen to minimize falsely elevated pH results, this task was thought to be more challenging for self-testing. Our results proved otherwise. The brief self-sampling instructions perhaps contributed to retrieving optimal specimens. Women can also be educated to avoid sampling after sexual intercourse and while menstruating to minimize spurious results. Our concern about how women sample for pH within the vagina may be tempered by these results that indicate little significant variation of intravaginal pH using simple sampling devices.

Recently, an over-the-counter vaginal self pH test was approved for use by the FDA.[19,24] However, this device is considerably more expensive and does not use a swab for sampling purposes. Instead, a small strip of pH paper mounted at the end of the sampler is used to obtain the specimen although the pH paper is considered for in vitro use only. Further, the pH scale may not adequately reflect a reasonable range of intravaginal pH because it only records values ≥4.5. Our pH paper values ranged from 3.0 to 5.5, more closely approximating usual normal or abnormal vaginal pH results.

One limitation of our study was that it included only asymptomatic women. It would be interesting to repeat our study in a population of symptomatic women to determine variation of pH within the abnormal vagina. One goal of this study was to determine whether a clinician could detect the normal intravaginal pH gradient using a swab to pH paper technique. This gradient would not exist in patients with an elevated pH as seen with bacterial vaginosis and Trichomonas vaginitis. A study of women with abnormal vaginal discharge should evaluate clinician/patient pH test variability and subsequent decision making as to the need of additional assessment by a health care provider. If symptomatic women perform similarly to clinicians, the medical community would be more confident in endorsing self-sampling of vaginal pH.

Our findings have important clinical relevance for women's health care. First, women are able to self-sample the vagina and measure intravaginal pH. Self-sampling and testing empowers women to become more involved with their medical care. The less intrusive technique can be done in private to minimize embarrassment. Furthermore, sampling can occur at a time suitable for women and not necessarily when convenient for a health care provider. More importantly, women's vaginal pH results were very similar to those measured by a medical provider. This concordance extends the examination room and laboratory to accommodate women in countless locations. Knowledge of intravaginal pH can guide women before selecting to inappropriately use over-the-counter products for vaginitis. Very simply, in symptomatic women, a high vaginal pH result would require further evaluation (if not premenarchal or postmenopausal) by a health care provider. A normal pH in symptomatic women would suggest vulvovaginal candidiasis prompting more selective use of an over-the-counter product. A better informed self-diagnosis would ultimately reduce individual financial expenditures, delayed treatment, and possible secondary complications.[3,6] It would also lower health care cost for the medical industry.[25] Based on our results, self-sampling of vaginal pH seems very suitable for implementation and should help improve health care for women.[26]

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