

## CLINICAL STUDY

# Diagnosis of bacterial vaginosis from self-obtained vaginal swabs

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### Abstract

**Objective:** To determine the concordance between vaginal fluid Gram stains and pH obtained at speculum exam with similar stains and pH prepared from self-obtained vaginal swabs.

**Methods:** Using vaginal fluid Gram stain, 129 pregnant women were screened for bacterial vaginosis at 24 to 29 weeks' gestation. Two smears were collected from each woman during the same prenatal visit: the first was prepared from a self-obtained vaginal swab and the second from a physician-obtained speculum examination. Vaginal pH was recorded for each swab. Kappa coefficient was used to quantify agreement between the two sets of results.

**Results:** When compared with the physician-obtained smear, the ability of the self-obtained Gram stain to diagnose bacterial vaginosis had a sensitivity of 77%, specificity of 97%, positive predictive value of 71% and negative predictive value of 97%. There was substantial agreement (weighted kappa = 0.82) between the two techniques in the ability to determine the grade of vaginal flora.

**Conclusion:** When compared with physician-obtained vaginal smears, self-obtained smears have substantial agreement in the diagnosis of bacterial vaginosis.

**Keywords:** *Bacterial vaginosis, self-obtained diagnosis*

### Introduction

Bacterial vaginosis is present in up to 23% of women during pregnancy [1]. A recent meta-analysis concluded that treatment of bacterial vaginosis in pregnancy was effective at eradicating bacterial vaginosis, but not at reducing the attendant risk of preterm birth [2]. However, among the subgroup of women with a previous preterm birth, the odds ratio for preterm birth was 0.37 (95% confidence interval (CI) 0.23 to 0.60). This meta-analysis did not include a recent large randomized controlled trial, which found no difference in the rates of preterm birth between the treatment and placebo groups overall nor in subgroups [3]. In view of this information, in 2001 the American College of Obstetricians and Gynecologists recommended that there are no current data to support the use of bacterial vaginosis screening as a strategy to prevent or identify preterm birth [4]. Since this recommendation, however, a large randomized trial involving 494 women has examined the effect of early oral

clindamycin on late miscarriage and preterm delivery in asymptomatic women with bacterial vaginosis [5]. This study found that treatment of asymptomatic bacterial vaginosis with oral clindamycin significantly reduced the rate of late miscarriage and spontaneous preterm birth in a general obstetric population. In order to make progress in this important line of research, it may well be necessary to assess bacterial vaginosis frequently throughout the course of pregnancy, since changes in bacterial vaginosis are fairly common [6]. Such evaluation calls for acceptable methods for the collection of specimens, with minimal expense and discomfort for the patient.

The diagnostic criteria established by Amsel et al. are simple and useful in clinical practice [7]. The Amsel criteria define bacterial vaginosis as being present if three of the four following criteria are met: homogeneous vaginal discharge, vaginal pH greater than 4.5, positive "whiff" test, and the presence of clue cells on wet microscopy of the vaginal fluid. This diagnostic scheme is limited, however, by its inherently subjective criteria and the need for

speculum examination. In fact, it may lead to the underdiagnosis of bacterial vaginosis [8].

A Gram stain of vaginal fluid is a reproducible test for the diagnosis of bacterial vaginosis [9, 10]. Nugent et al. [9] described a Gram-stain method for the diagnosis of bacterial vaginosis which is being used increasingly for large studies [11, 12]. This approach also requires examination with a speculum to obtain specimens. If such screening were to become more routine in the antenatal clinic, the test would need not only to be reliable and reproducible but also non-invasive and easy to perform. In addition, self-obtained swabs may be useful in longitudinal studies examining the natural history of bacterial vaginosis. Self-obtained vaginal swabs have been used successfully for the diagnosis of several vaginal infections, including group B streptococcus [13], *Chlamydia trachomatis* [14], tricho-monas [15] and bacterial vaginosis [13, 16, 17]. To our knowledge, no studies have examined the ability of vaginal pH to be obtained from self-obtained vaginal swabs.

The purpose of this study was to determine the correlation between vaginal fluid Gram stains and vaginal pH obtained at speculum exam with those prepared from self-obtained vaginal swabs.

## Materials and methods

This study is part of an observational cohort study of pregnant women who received prenatal care at the University of North Carolina Hospitals. Eligible women were at least 16 years old, made prenatal visit(s) before enrollment at 24 to 29 weeks' gestation, spoke English, and carried single fetuses. The study was approved by the institutional review board, and informed consent was obtained at the time of recruitment. Women who had given informed consent were screened for bacterial vaginosis and asked to obtain a self-collected vaginal swab before the speculum examination usually performed for screening purposes.

Between 27 October 1997 and 11 May 1998, 129 pregnant women were screened for bacterial vaginosis at 24 to 29 weeks' gestation. Two smears were collected from each participant during the same prenatal visit; the first was prepared from a self-obtained vaginal swab and the second from a physician-obtained speculum examination. Each smear was evaluated using vaginal fluid Gram stain and pH.

The self-obtained smears were obtained as follows. The woman was instructed to insert a Dacron-tipped swab into her vagina to a pre-marked depth of 7.5 cm, and to press the swab against the vaginal wall while rotating it a few times. The swab was then withdrawn, wiped onto a numbered microscope slide and allowed to air dry. The participant then touched

the swab to a pH indicator strip (4.0 to 7.0). The research nurse promptly compared the pH strip with a color chart and the results were recorded. The identity of the participant was recorded by the research nurse but was not revealed until all the smears had been examined microscopically. The participant then proceeded directly to the physician for the usual screening examination.

The physician-obtained smears were obtained as follows. A non-lubricated speculum was passed into the vagina, a Dacron-tipped swab was inserted and a sample taken from the vaginal wall of the upper third of the vagina. The swab was then wiped onto a numbered microscope slide and allowed to air dry. Vaginal pH was measured by touching the collection swab to a pH indicator strip (4.0 to 7.0). A second nurse, who was blinded to the pH reading from the self-obtained sample, promptly compared the pH strip with a color chart and recorded the result.

All smears were Gram stained on an automated processor (Laboratory Corporation of America, Burlington, NC, USA). Vaginal microflora was classified according to the Nugent score(9): 0 to 3 = grade 1 (normal); 4 to 6 = grade 2 (intermediate); 7 to 10 = grade 3 (bacterial vaginosis). All the smears were reviewed by a trained examiner (J.I.F.) who was blinded to any other information about the participant.

The kappa coefficient was used to correlate the two sets of results. The kappa statistic ranges from 0 to 1, with 1 representing perfect agreement and 0 representing no more agreement than would be expected by chance alone. The weighted kappa statistic(18) was used to measure agreement when multiple categories were analyzed. The weighted kappa statistic gives no credit for agreement equal to that expected by chance alone but gives partial credit for responses that are in close but not perfect agreement. In this study, perfect agreement was given a weight of 1, and the remaining weights were determined by using the default in our statistical software (weights  $1 - |i - j| / (k - 1)$ , where  $i$  and  $j$  index the rows and columns of the ratings by the two raters and  $k$  is the maximum number of possible ratings). Calculated kappa values of  $\leq 0.40$  are considered to reflect poor to fair reproducibility or agreement, those between 0.40 and 0.80 are considered to reflect moderate to substantial agreement, and those of  $> 0.80$  reflect almost perfect agreement [19]. Data were analyzed using SAS 6.12 (SAS Institute, Cary, NC, USA) and STATA 7.0 (Stata Corporation, College Station, TX, USA).

## Results

The results, comparing vaginal microflora between the two techniques, are shown in Table I. The prevalence of bacterial vaginosis diagnosed by the

physician-obtained swabs was 10%. The results for 122 of the 129 smears were concordant for the correct diagnosis of bacterial vaginosis for an overall concordance of 95% (Table I). There was substantial agreement ( $\kappa = 0.71$ ; 95% CI 0.51 to 0.91) between the two techniques. When compared with the physician-obtained smear, the ability of the self-obtained smear to diagnose bacterial vaginosis had a sensitivity of 77%, specificity of 97%, positive predictive value of 71% and negative predictive value of 97%.

The results of comparing dichotomous coding of vaginal pH (greater than 4.5 versus less than or equal to 4.5) between the two techniques are shown in Table II. Of the 129 self-obtained vaginal pH samples, 7 (5%) were uninterpretable because an inadequate amount of vaginal fluid was placed on the nitrazine paper. Thus, 122 results were available for comparison. There was moderate agreement ( $\kappa = 0.49$ ; 95% CI 0.32 to 0.65) between the two techniques of obtaining vaginal pH. When compared with the physician-obtained smear, the ability of the self-obtained swab to identify a vaginal pH > 4.5 had a sensitivity of 49%, a specificity of 95%, a positive predictive value of 83%, and negative predictive value of 78%.

The comparison between the two techniques was repeated using both Nugent score and vaginal pH. Using this definition, 7 of 129 samples were uninterpretable (5%). For the 122 vaginal smears with interpretable pH and Nugent score information,

Table I. \*Comparison of Gram-stained vaginal fluid collected by the two techniques using dichotomous coding of vaginal flora score.

Self-obtained vaginal flora scores	Physician-obtained vaginal flora scores		
	0-6	7-10	Total
0-6	112	3	115
7-10	4	10	14
Total	116	13	129

\*Nugent score 0 to 6, normal and intermediate; Nugent score 7 to 10, bacterial vaginosis;  $\kappa = 0.71$  (95% confidence interval 0.51 to 0.91).

Table II. \*Comparison of dichotomous coding of vaginal fluid pH collected by the two techniques.

Vaginal pH of the patient-obtained specimen		Vaginal pH of the physician-obtained specimen		
		≤ 4.5	> 4.5	Total
Vaginal pH of the patient-obtained specimen	≤ 4.5	77	21	98
	> 4.5	4	20	24
Total		81	41	122

\*Kappa = 0.49 (95% confidence interval, 0.32 to 0.65).

there was 74% agreement (weighted kappa = 0.58; 95% CI 0.44 to 0.71) for the diagnosis of bacterial vaginosis as displayed in Table III.

The vaginal flora scores of samples obtained by the participant and by the physician were cross-tabulated into three grades (0 to 3 = grade 1, 4 to 6 = grade 2, 7 to 10 = grade 3) and compared. The results are displayed in Table IV. There was substantial agreement (weighted kappa = 0.82; 95% CI 0.72 to 0.91) between the two techniques in the ability to determine the grade of vaginal microflora.

## Discussion

Blind swabbing of the vagina appears to have a high degree of acceptability to women and they can perform it themselves. Our data demonstrate that with specific instructions and a marked swab to help assure adequate depth of sampling, self-obtained swabs can reasonably but not perfectly approximate specimens obtained by clinicians during speculum examination.

Other authors have studied the ability of vaginal swabs to diagnose bacterial vaginosis [16, 17, 20, 21], but none of these studied self-obtained specimens from a pregnant population. Although two studies [13, 22] have examined self-obtained collection in a pregnant population, our study is unique in the inclusion of vaginal pH (collected by self-obtained swab) to diagnose bacterial vaginosis. Among 88 pregnant women, Morgan et al. [21] found almost perfect agreement ( $\kappa = 0.85$ ) between Gram-stained smears prepared from physician-obtained blind vaginal swabs with those obtained at speculum examination for the assessment of vaginal flora. Strum et al. [22] evaluated tampon fluid preparations and vaginal smears collected during speculum examination using the Nugent score for 84 pregnant women. They found excellent agreement (Spearman's coefficient > 0.80) between these two methods in the diagnosis of bacterial vaginosis. Schwebke et al. [16] found good correlation (Spearman's correlation coefficient  $r = 0.74$ ) between self- and clinician-obtained pairs of smears among 18 non-pregnant women, but they did not obtain the vaginal pH.

In view of the biologic relevance of alterations in the vaginal microflora to women of reproductive age [12], a self-obtained diagnostic technique that allows clinicians and patients to forego speculum examination is attractive. If diagnosis and treatment of bacterial vaginosis is ultimately found to prevent preterm birth [5], this diagnostic approach has potentially important use in screening under-served or rural obstetrical populations. Self-obtained vaginal swabs could be performed at home and mailed to a healthcare provider. In addition, observational and randomized trials studying bacterial vaginosis could

Table III. \*Comparison of both Gram-stained vaginal fluid and vaginal pH collected by the two techniques.

Self-obtained Nugent score and pH	Clinician-obtained specimen, Nugent score and vaginal pH				Total
	7–10, > 4.5	7–10, ≤ 4.5	0–6, > 4.5	0–6, ≤ 4.5	
7–10, > 4.5	6	1	4	0	11
7–10, ≤ 4.5	1	1	0	0	2
0–6, > 4.5	2	0	8	3	13
0–6, ≤ 4.5	0	1	20	75	96
Total	9	3	32	78	122

\*Simple kappa = 0.43 (95% confidence interval, 0.29 to 0.57); weighted kappa = 0.58 (95% confidence interval, 0.44 to 0.71).

Table IV. \*Comparison of Gram-stained vaginal fluid collected by the two techniques using vaginal flora scores stratified into 3 levels (grade 1, normal, 0–3; grade 2, intermediate, 4–6; grade 3, bacterial vaginosis, 7–10).

Self-obtained vaginal swab	Physician-obtained vaginal swab			Total
	Grade 1	Grade 2	Grade 3	
Grade 1	89	1	1	91
Grade 2	5	17	2	24
Grade 3	0	4	10	14
Total	94	22	13	129

\*Simple kappa = 0.77 (95% confidence interval 0.66 to 0.88); weighted kappa = 0.82 (95% confidence interval 0.72 to 0.91).

use patient-obtained vaginal smears for the diagnosis of bacterial vaginosis, and could expand the protocols to include multiple evaluations during the course of pregnancy.

Our work is limited by small numbers, a reliance on verbal rather than written instructions, and the evaluation of self-collection in a highly motivated population of research subjects. Each limitation reduces any claims we can make about generalizability and the usefulness of widespread self-collection. Nevertheless, our results are encouraging and, if duplicated, could have important implications for the diagnosis of bacterial vaginosis, particularly in view of the recent evidence demonstrating a reduction in late miscarriage and spontaneous preterm birth associated with treatment of asymptomatic bacterial vaginosis [5].

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