

## Gram Stain Method Shows Better Sensitivity Than Clinical Criteria for Detection of Bacterial Vaginosis in Surveillance of Pregnant, Low-Income Women in a Clinical Setting

M.T. Tam,<sup>1\*</sup> M. Yungbluth,<sup>2</sup> and T. Myles<sup>3</sup>

<sup>1</sup>Department of Obstetrics and Gynecology, St. Joseph Hospital, Chicago, IL

<sup>2</sup>Department of Pathology, St. Joseph Hospital, Chicago, IL

<sup>3</sup>Maternal-Fetal Medicine, St. Joseph Hospital, Chicago, IL

### ABSTRACT

**Objective:** The purpose of the study is to determine whether the Gram stain method is superior to the clinical criteria for the diagnosis of bacterial vaginosis in low-income pregnant women seen in a resident clinic setting. The clinical criteria is the current diagnostic method employed to diagnose bacterial vaginosis.

**Study Design:** In this study, 51 pregnant women with vaginal discharge were prospectively evaluated. All were screened using the clinical criteria, Gram stain method, and culture of the discharge. The modified scoring system instituted by Nugent et al. (J Clin Microbiol 29:297–301, 1991) was employed in reading the Gram stain smears. The clinical criteria were then compared with the Gram stain method. Isolation of moderate to many *Gardnerella vaginalis* growth by culture was used as the confirmatory finding.

**Results:** Sensitivity of the Gram stain method (91%) was significantly higher than that of the clinical criteria (46%), (sign test  $P = 0.0023$ ,  $<0.01$ ). The Gram stain method also has both a low false-negative (4%) and high negative predictive value (96%), making it an ideal diagnostic test.

**Conclusion:** The Gram stain method is a rapid and cost-effective test that is also highly reproducible and readily available in many laboratories. These features make the Gram stain method a more desirable screening procedure for bacterial vaginosis in a clinic population. Infect. Dis. Obstet. Gynecol. 6:204–208, 1998. © 1998 Wiley-Liss, Inc.

### KEY WORDS

*Gardnerella vaginalis*; pregnancy; vaginal discharge

**B**acterial vaginosis is the most common vaginal infection among reproductive-aged women.<sup>1</sup> In this condition, the normal lactobacillus-dominant vaginal flora is replaced by a microflora with disproportionate numbers of *Gardnerella vaginalis*, anaerobic bacteria (*Mobiluncus*, *Prevotella*, *Porphyromonas*, and *Bacteroides* species), and *Mycoplasma* species.<sup>2,3</sup>

Affecting 12–22 percent of pregnant women,<sup>1</sup> this altered vaginal microbial ecology is asso-

ciated with increased risk of preterm labor, premature rupture of membranes, chorioamnionitis, and delivery of low birthweight infants independent of other risk factors.<sup>4–8</sup> Postpartum endometritis and pelvic inflammatory disease have been attributed to bacterial vaginosis.<sup>9,10</sup> Early identification of bacterial vaginosis and appropriate antibiotic intervention can reduce the likelihood of adverse pregnancy outcome.<sup>11,12</sup>

\*Correspondence to: Dr. Maria Teresa Tam, Department of Obstetrics and Gynecology, St. Joseph Hospital, Chicago, IL

Several methods are available for diagnosis of bacterial vaginosis. Gas-liquid chromatography is a method employed to analyze vaginal fluid for short-chain fatty acids (metabolic products of anaerobic bacteria),<sup>13</sup> but it is not widely available to all laboratories. One could also obtain a culture from the vaginal discharge, but awaiting bacterial growth followed by bacterial identification are both labor intensive and time consuming. The current clinical criteria is the presence of three out of the four following clinical observations: vaginal pH > 4.5, presence of clue cells, positive potassium hydroxide (KOH) whiff test, and homogeneous, malodorous discharge.<sup>14</sup> Interpretation of all but the pH level is subjective, resulting in greater interobserver variability and inconsistency in the diagnosis of bacterial vaginosis. Gram stain of the vaginal discharge has been shown to be reproducible for the diagnosis of bacterial vaginosis<sup>15,16</sup> and readily available in many laboratories. Current studies have suggested that the clinical criteria could lead to underdiagnosis of bacterial vaginosis.<sup>17</sup> This study prospectively compared Gram stain of vaginal discharge in pregnant women with the clinical criteria. Isolation of *G. vaginalis* by the conventional culture method was used as the confirmatory finding.

#### MATERIALS AND METHODS

During the study period, August 1996 to August 1997, pregnant women with vaginal discharge were screened for bacterial vaginosis. Fifty-one symptomatic but otherwise healthy pregnant women were enrolled in the study. These women obtained prenatal care at the Labouré Clinic of Saint Joseph Hospital in Chicago, IL.

A cotton swab was used to obtain the discharge from the vaginal walls and smeared onto two glass slides. The slides were air-dried, labeled with the patient's name, and placed in a slide holder for transport to the laboratory. A clinical evaluation sheet noting the patient's name, medical record number, date of exam, and gestational age was filled out by the resident providing patient care. It also included a checklist of clinical findings according to the clinical criteria. The evaluation sheet together with the vaginal smears were sent to the microbiology laboratory for Gram stain and evaluation.

Gram stain was performed using safranin as the

TABLE 1. Scoring system (0–10) for Gram-stained vaginal smears<sup>a</sup>

Score <sup>b</sup>	<i>Lactobacillus</i> morphotypes	<i>Gardnerella</i> and <i>Bacteroides</i> spp.	Curved gram variable rods
0	4+	0	0
1	3+	1+	1+ or 2+
2	2+	2+	3+ or 4+
3	1+	3+	
4	0	4+	

<sup>a</sup>Morphotypes are scored as the average number seen per oil immersion field. Note that less weight is given to curved gram-variable rods. Total score = *Lactobacilli* + *G. vaginalis* and *Bacteroides* spp. + curved rods.

<sup>b</sup>0, No morphotypes present; 1, <1 morphotype present; 2, 1 to 4 morphotypes present; 3, 5 to 30 morphotypes present; 4, 30 or more morphotypes present.

counterstain and evaluated under oil immersion (1,000×). Only one pathologist who was blinded to the clinical findings performed the Gram stain interpretation to ensure the reproducibility of the Gram stain finding. Both smears were read to provide a general overview of the vaginal microflora. Having two vaginal smear slides also provided a back-up smear in case of handling mishaps or Gram stain procedure problems. Gram stain interpretation and scoring were done using the method proposed by Nugent et al.<sup>18</sup> (Table 1).

Quantitation of each bacterial morphotype was performed by reviewing at least three oil immersion fields. The number of each bacterial morphologic type from 1 to 4+ in a typical oil immersion field was counted and an average was obtained for scoring. The scores of all three bacterial morphotype categories were added and a final score obtained. Final scores of 7–10 suggested bacterial vaginosis, 4–6 was intermediate, and 0–3 was a normal smear result. During reviews of the Gram stain, the presence of neutrophils and acute inflammation (mild, moderate, or severe) and the presence of yeast were also noted on the worksheet.

Using the clinical criteria, a positive diagnosis required three of the four clinical findings: elevated vaginal pH (> 4.5), presence of clue cells on wet-mount microscopy, amine odor with KOH alkalization, and a thin, gray, homogeneous, malodorous vaginal discharge. Vaginal pH level was determined by placing the pH paper directly on the vaginal wall or in the vaginal discharge. A pH level greater than 4.5 was associated with amine residue released from proteolytic anaerobic bacterial metabolism. The whiff test was done by plac-

**TABLE 2.** Results of clinical criteria, Gram stain, and culture methods

Screening method	Negative	Positive	Other	N
Clinical criteria	42	9	-	51
Gram stain	27	13	11 <sup>a</sup>	51
Culture	34	13	4 <sup>b</sup>	51

<sup>a</sup>Intermediate Gram stain finding.<sup>b</sup>Missing culture results.

ing a drop of vaginal discharge on a glass slide and adding a drop of 10% KOH. A fishy odor from the liberation of volatile amines was noted from anaerobic bacterial overgrowth. Microscopic examination of the vaginal discharge through a wet-mount (100–200 $\times$ ) revealed presence of clue cells (squamous epithelial cells studded with coccobacillary organisms). Wet-mount microscopy also identified *Trichomonas* flagellates or yeast pseudohyphae.

Vaginal culture was obtained from the vaginal discharge on symptomatic women and isolated into a sheep blood agar plate (BAP). The BAP was incubated at 37° C in 5% CO<sub>2</sub> in air. After 48–72 hr of incubation, *G. vaginalis* was identified as follows: small beta-hemolytic colonies on BAP, pleomorphic gram-variable coccobacilli on Gram stain, negative catalase test, and positive alpha inhibition test.<sup>19</sup> Only cultures with moderate to many growth of *G. vaginalis* were reported as positive.

Frequencies and percentages were computed for clinical criteria, Gram stain tests, and cultures. Sensitivity, specificity, false-negative and false-positive, and positive or negative predictive values were determined for clinical criteria and Gram stain tests when compared with culture.

## RESULTS

Clinical criteria was compared with the Gram stain method for the diagnosis of bacterial vaginosis in 51 symptomatic pregnant women. Among the 51 women evaluated, 42 were diagnosed as negative and nine were positive based on clinical criteria. According to the Gram stain method, 27 were deemed negative, 11 had intermediate findings (scores between 4 to 6) and 13 were positive (Table 2).

Table 3 shows the comparison of the clinical criteria with the Gram stain method with respect to the culture finding. Isolation of *G. vaginalis* on culture was used as the final diagnostic criteria. In

addition to giving a final Gram stain score, a descriptive impression of the smear, such as presence of neutrophils and yeast, was mentioned if relevant.

All of the 11 women with intermediate Gram stain findings had abnormal vaginal flora with neutrophils present consistent with acute vaginitis. Six of the 11 women with intermediate Gram stain results did not grow *G. vaginalis* on culture but grew other types of microorganisms. Two women grew yeast, three had group B beta-hemolytic streptococci, and one had group F beta-hemolytic streptococci, all of which were isolated on culture. Four out of the 51 women did not have genital cultures due to a computer ordering error. Three out of the four women without genital cultures had intermediate Gram stain results. Out of these three women, two were treated for yeast infections. All women with yeast infections were initially diagnosed based on Gram stain and treated while awaiting culture results. Women without genital cultures and intermediate Gram stain results were not included in the statistical analysis of the data.

The isolation of *G. vaginalis* in culture was used as the definitive test. The Gram stain method was more sensitive (91%) than the clinical criteria (46%). Although the specificity, false-positive, and positive predictive values between clinical criteria and the Gram stain methods were essentially similar, the Gram stain method revealed a lower false negative rate (4%) and a higher negative predictive value (96%) when compared with the clinical criteria (Table 4).

There was strong evidence to suggest that Gram stain has significantly better sensitivity than the clinical criteria. The sign test (exact test) had been performed. This resulted in a two-tail *P* value = 0.0023. A McNemar's test for correlated proportions-exact test for the same data gave the two-tailed *P* value = 0.004. Both *P* values are much smaller than the  $\alpha = 0.05$  significance level.

## DISCUSSION

The results of this study support the use of the Gram stain method in diagnosing bacterial vaginosis. Using the clinical criteria, sensitivity is compromised by the subjectivity of the clinical finding. Other laboratory methods, such as gas liquid chromatography, or the more recent, proline aminopeptidase test,<sup>20</sup> are highly specialized procedures and

TABLE 3. Comparison of clinical criteria with Gram stain in relation to culture result

Isolation of <i>G. vaginalis</i> by culture	Clinical criteria		Gram stain method		
	Negative n = 42	Positive n = 9	Negative n = 27	Intermediate n = 11	Positive n = 13
Negative (n = 34)	32	2	25	6	3
Positive (n = 13)	7	6	1	2	10
No culture (n = 4)	3	1	1	3	0

TABLE 4. Comparison of the sensitivity, specificity, false-negative, false-positive, and predictive values between clinical criteria and Gram stain method<sup>a</sup>

Method/total no. of observations	Sensitivity	Specificity	False positive	False negative	Positive predictive value	Negative predictive value
Clinical criteria n = 46	0.46	0.94	0.25	0.18	0.75	0.82
Gram stain n = 39	0.91	0.89	0.23	0.04	0.77	0.96

<sup>a</sup>Sign test.  $P = 0.0023$ ,  $< 0.01$ .

as a result are not readily available to many laboratories. Studies in pregnant women would also be needed using these confirmatory techniques.

Specific aerobic or anaerobic vaginal cultures are not indicated to establish the diagnosis of bacterial vaginosis. Since bacterial vaginosis is characterized by a predominance of mixed anaerobic flora, genital culture of the vaginal discharge would isolate several types of bacteria. Individual isolation of each bacterial morphotype would be prohibitively costly and time consuming. Semi-quantitation of the different colony types in culture as described by Rosenstein et al.<sup>21</sup> could possibly be a better confirmatory method, but time and monetary constraints only allowed for the semi-quantitation of *G. vaginalis* in culture.

Gram stain evaluation correlates well with clinical diagnosis and presents a more reliable and reproducible method of diagnosing bacterial vaginosis.<sup>22</sup> It is also inexpensive and widely available to many laboratories. The Gram stain technique could be used to screen symptomatic pregnant women with underlying acute vaginitis who may otherwise be negative by clinical criteria. The Gram stain method allows the interpreter to identify other associated findings, such as the presence of yeast or neutrophils seen with acute vaginitis.

Bacterial vaginosis has been associated with adverse pregnancy outcomes.<sup>23</sup> These risks are compounded with the other associated risk factors evident with patients in a clinic population. By allowing improved and early diagnosis of the presence of bacterial vaginosis, prompt treatment can be insti-

tuted for genital infections in pregnancy. Early diagnosis and intervention in symptomatic women could prevent complications. The rapidity, specificity, and sensitivity of the Gram stain technique for the diagnosis of bacterial vaginosis could potentially improve pregnancy outcomes. All these together with a low false-negative rate make the Gram stain an excellent diagnostic method for the detection of bacterial vaginosis.

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