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Reliability of Diagnosing Bacterial Vaginosis Is Improved by a Standardized Method of Gram Stain Interpretation

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The purpose of the study was to examine intercenter variability in the interpretation of Gram-stained vaginal smears from pregnant women. The intercenter reliability of individual morphotypes identified on the vaginal smear was evaluated by comparing them with those obtained at a standard center. A new scoring system that uses the most reliable morphotypes from the vaginal smear was proposed for diagnosing bacterial vaginosis. This scoring system was compared with the Spiegel criteria for diagnosing bacterial vaginosis. The scoring system (0 to 10) was described as a weighted combination of the following morphotypes: lactobacilli, *Gardnerella vaginalis* or bacteroides (small gram-variable rods or gram-negative rods), and curved gram-variable rods. By using the Spearman rank correlation to determine intercenter variability, gram-positive cocci had poor agreement (0.23); lactobacilli (0.65), *G. vaginalis* (0.69), and bacteroides (0.57) had moderate agreement; and small (0.74) and curved (0.85) gram-variable rods had good agreement. The reliability of the 0 to 10 scoring system was maximized by not using gram-positive cocci, combining *G. vaginalis* and bacteroides morphotypes, and weighting more heavily curved gram-variable rods. For comparison with the Spiegel criteria, a score of 7 or higher was considered indicative of bacterial vaginosis. The standardized score had improved intercenter reliability ($r = 0.82$) compared with the Spiegel criteria ($r = 0.61$). The standardized score also facilitates future research concerning bacterial vaginosis because it provides gradations of the disturbance of vaginal flora which may be associated with different levels of risk for pregnancy complications.

Bacterial vaginosis is a syndrome marked by an increased vaginal pH, milky creamy discharge, and amine or fishy odor. Microbiologically, bacterial vaginosis is characterized by a shift in the vaginal flora from the dominant flora of *Lactobacillus* spp. to a mixed vaginal flora that includes *Gardnerella vaginalis*, *Bacteroides* spp., *Mobiluncus* spp., and *Mycoplasma hominis* (11, 12). Because bacterial vaginosis is a clinical syndrome which has been associated with a group of genital microorganisms rather than a single etiologic agent, it has been defined primarily by the following clinical signs: vaginal pH >4.5, the presence of adherent white discharge, detection of "clue cells," and the presence of an amine odor after the addition of KOH (1). Laboratory methods for the diagnosis of bacterial vaginosis have included culture for *G. vaginalis* (5, 13, 17), direct Gram stain of vaginal secretions (3, 15), biochemical tests for metabolic by-products of vaginal bacteria (gas chromatography) (14), and more recently, the proline aminopeptidase test (16).

In recent studies, bacterial vaginosis has been associated with amniotic fluid infection (6), histologic chorioamnionitis (8), postcesarean endometritis (18), and prematurity (7, 11). The risk of bacterial vaginosis to pregnant women should be verified in larger, more definitive studies. To conduct large, multicenter studies of bacterial vaginosis, standardized interpretive criteria that yield comparable results when performed by different microbiologists are necessary. Diagnostic criteria for bacterial vaginosis which provided for gradations in the severity of disease would allow the study of a dose-response relationship between bacterial vaginosis and pregnancy complications. A diagnostic method would be most valuable if it could also provide a permanent record of the patient specimen used for diagnosis.

Of the diagnostic methods currently available, assessment of clinical signs is the "gold standard," but the signs are subtle and detection of the signs is very dependent on the acuity of the clinician performing the test. The use of clinical signs for the diagnosis of bacterial vaginosis in a large study with more than one clinician would provide the problems of standardizing the observations and determining the comparability of results determined by various clinicians. Among the laboratory methods for the diagnosis of bacterial vaginosis, Gram-stained vaginal smears are the least expensive, require the least time to perform, and are more widely available than other laboratory methods are. However, this is the most interpretive of the laboratory methods. One study has shown that the Gram stain interpretation for diagnosis of bacterial vaginosis has a high intracenter reliability (12). No studies have yet determined the reproducibility of the diagnosis of bacterial vaginosis by a Gram-stained vaginal smear when used in different centers by different microbiologists. The permanent record of the bacterial morphotypes available on a smear makes it possible to address the question of the reliability of this method of diagnosis.

This study examined the intercenter variability in the interpretation of Gram-stained vaginal smears from pregnant women participating in a large multicenter study. The intercenter reliability was evaluated for the individual morphotypes identified. A new scoring system which allows gradations in severity and which uses the morphotypes that are most reliably identified at all centers was proposed for diagnosing bacterial vaginosis. The intercenter reliability of the Spiegel criteria for diagnosing bacterial vaginosis was

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compared with the reliability of the new scoring criteria for diagnosing bacterial vaginosis.

MATERIALS AND METHODS

The Vaginal Infection and Prematurity Study, which is sponsored by a multicenter contract from the National Institute of Child Health and Human Development and the National Institute of Allergy and Infectious Diseases, has assembled cohorts of women at five centers since 1984 to study the effects of genital flora during pregnancy on prematurity and other pregnancy complications. After 2 years of data collection (1984 to 1986), sufficient numbers of women were enrolled to assess the reliability of Gram stain interpretations of vaginal smears. Fifty smears of vaginal discharge were selected from each of the following five participating centers: Columbia University, New York, N.Y.; University of Washington, Seattle; University of Texas—San Antonio, San Antonio; University of Oklahoma, Oklahoma City; and Louisiana State University, New Orleans. The smears were selected from an available pool of 6,200 women who had enrolled in the study at between 23 and 26 weeks of gestation. The slides were first read at each site by a microbiologist who recorded the quantity of each morphotype (1 to 4+) and grouped the smears into categories: normal, bacterial vaginosis, inflammation, yeasts, and other. This first categorization of smears was a temporary grouping for the purpose of choosing smears from each center. Fifty slides were chosen from the cohort at each center by a sampling fraction which yielded approximately equal numbers of slides indicating normal and bacterial vaginosis smears, while all slides within the other categories, which were rarely used, were selected. Each of the 50 smears was read again by a microbiologist at the original center and then by a microbiologist at the reference laboratory (University of Washington), each of whom recorded the number of individual morphotypes (1 to 4+ for each morphotype) on a uniform data collection instrument. The center identifiers were masked before the slides were delivered to the reference laboratory. Among the 200 slides from other centers that were read at the reference center, 3 slides were broken in transit and 5 had incomplete data, leaving 192 to 194 slides available for analysis, depending on the individual morphotype.

At each center, during the enrollment visit between 23 and 26 weeks of gestation, the women were given a vaginal speculum examination without lubrication. The vaginal smear for subsequent Gram staining was obtained after the assessment of clinical signs and before the vaginal specimens were taken for the isolation of bacteria. A vaginal smear was obtained by rolling a swab across the vaginal wall and then onto a glass slide. The smears were heat fixed and Gram stained by using safranin as the counterstain.

Each Gram-stained smear was evaluated for the following morphotypes under oil immersion (×1,000 magnification): large gram-positive rods (lactobacillus morphotypes), small gram-variable rods (*G. vaginalis* morphotypes), small gram-negative rods (*Bacteroides* spp. morphotypes), curved gram-variable rods (*Mobiluncus* spp. morphotypes), and gram-positive cocci. Fusiform rods and gram-negative cocci were also noted, but only two slides in this set were positive, so no comparisons were made. Each morphotype was quantitated from 1 to 4+ with regard to the number of morphotypes per oil immersion field (0, no morphotypes; 1+, less than 1 morphotype; 2+, 1 to 4 morphotypes; 3+, 5 to 30 morphotypes; 4+, 30 or more morphotypes) by a microbiologist who was unaware of the clinical or microbiological findings for these women.

TABLE 1. Scoring system (0 to 10) for Gram-stained vaginal smears[a]

Score[b]	Lactobacillus morphotypes	*Gardnerella* and *Bacteroides* spp. morphotypes	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+	

[a] 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.

[b] 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.

The quantitated morphotypes were assessed by two different criteria for the diagnosis of bacterial vaginosis. Bacterial vaginosis was diagnosed by (i) the Spiegel criteria and (ii) a scoring system from 0 to 10 developed by the Vaginal Infection and Prematurity study group. Bacterial vaginosis was present by the Spiegel criteria if lactobacillus morphotypes were fewer than five per oil immersion field and if there were five or more *G. vaginalis* morphotypes together with five or more other morphotypes (gram-positive cocci, small gram-negative rods, curved gram-variable rods, or fusiforms) per oil immersion field. If five or more lactobacilli and fewer than five other morphotypes were present per oil immersion field, the Gram stain was considered to be normal by the Spiegel criteria. The criteria developed by the Vaginal Infection and Prematurity study group used a scoring system from 0 to 10 which allowed for gradations in the severity of bacterial vaginosis and used only those morphotypes which had good intercenter agreement. The scoring criteria summed the weighted quantitation (0, 1 to 4+) of the following morphotypes to yield a score of 0 to 10 for each person: large gram-positive rods (lactobacillus morphotypes) (weighted such that absence yielded the highest score), small gram-negative to -variable rods (*G. vaginalis* and *Bacteroides* spp. morphotypes), and curved gram-negative rods (*Mobiluncus* spp. morphotypes) (Table 1). The criterion for bacterial vaginosis was a score of 7 or higher; a score of 4 to 6 was considered intermediate, and a score of 0 to 3 was considered normal (Fig. 1).

Intercenter reliability was determined for each individual morphotype, for several combinations of morphotypes, for the Spiegel criteria for diagnosing bacterial vaginosis, and for the scoring system (0 to 10) criteria of bacterial vaginosis. For the individual morphotypes, intercenter reliability was evaluated both for the 1 to 4+ quantitation and for the presence or absence of the morphotype. Statistical analyses were done by using the Pearson product moment correlation coefficient for dichotomous data and a combination of the Spearman rank correlation coefficient, mean differences in category scored, and the paired *t* test for polychotomous data. While the rank correlation coefficient allows for a comparison of the relative scoring of the two readings, it cannot detect systematic shifts of one or more categories by different readers. To detect such systematic shifts, the scores assigned by the reference center were subtracted from those assigned by the original reading center. The mean differences in the category scored were then tested by the

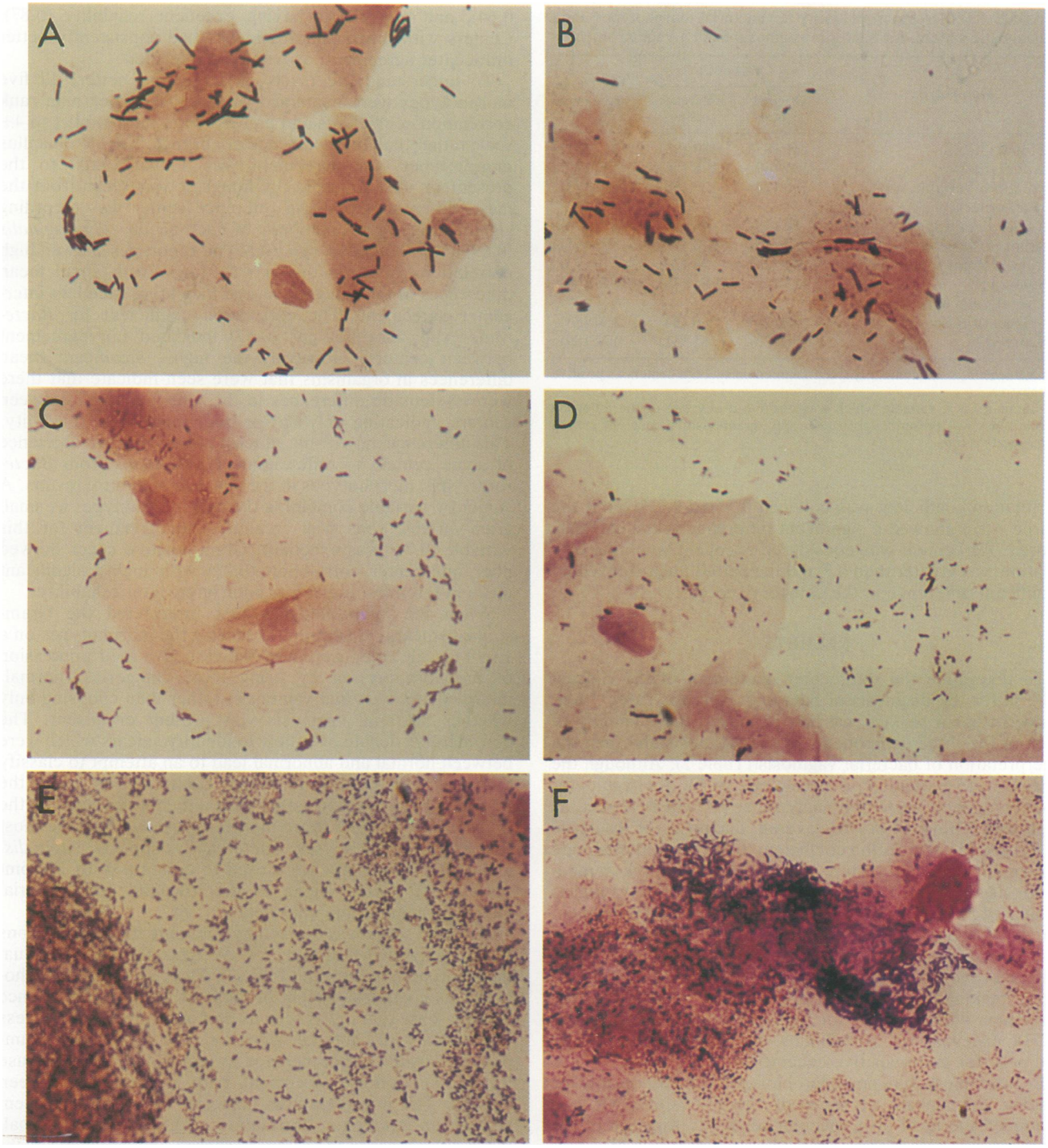

FIG. 1. Gram-stained vaginal smears from women with normal vaginal flora (A and B), intermediate vaginal flora (C and D), or bacterial vaginosis (E and F). (A) The 4+ lactobacillus morphotypes, no small gram-negative or gram-variable rods (score = 0); (B) 3+ lactobacillus morphotypes, 1+ *Gardnerella* spp. morphotypes (score = 2); (C) 3+ lactobacillus morphotypes and 3+ small gram-variable rods (score = 4); (D) 2+ lactobacillus morphotypes and 4+ small gram-negative and -variable rods (score = 6); (E) no lactobacilli and 4+ gram-negative and -variable rods (score = 8); note the margin of clue cells on the left; (F) no lactobacilli and 4+ gram-negative rods and curved rods (score = 10); note the *Mobiluncus* spp. morphotypes on the clue cell (center of field).

paired *t* test to determine whether the difference in the category scored was significant. These methods were used in addition to percent agreement, because percent agreement does not account for chance agreement, nor is it useful for data which have more than two ordered categories (2, 10).

By using criteria similar to those established for the kappa statistic, agreement was considered to be poor when the correlation coefficient was below 0.5 or the paired *t* test showed a significant difference between the categories scored (4). Agreement was considered moderate when the

TABLE 2. Intercenter reliability for bacterial morphotypes and diagnostic criteria for bacterial vaginosis from a vaginal smear

Morphotype	No. (%) positive		No. (%) in agreement[a]
	Original center	Reference center	
Lactobacilli	161 (83)	180 (93)	157 (81)
G. vaginalis	134 (69)	128 (66)	150 (77)
Small gram-variable rods	135 (70)	127 (66)	151 (78)
Spiegel criteria			
Bacteroides spp.	59 (30)	46 (24)	145 (75)
Gram-positive cocci	83 (43)	138 (72)	97 (51)
Curved gram-variable rods	44 (23)	46 (24)	179 (93)
Summary[b]	115 (59)	94 (48)	128 (66)
Bacterial vaginosis			
Spiegel criteria	56 (29)	66 (34)	160 (83)
Score ≥7	56 (29)	61 (31)	172 (89)

[a] Agreement is the number (percent) agreed on by both centers as positive or negative.

[b] Summary is a variable which was positive if any one of the following morphotypes was present: *Bacteroides* spp., gram-positive cocci, or curved gram-negative rods.

correlation coefficient was between 0.5 and 0.7, even if the paired *t* test showed a significant difference in the category scored. Agreement was considered excellent when the correlation was greater than 0.7 and the paired *t* test showed no significant difference in the category scored.

RESULTS

To determine the intercenter agreement of the Spiegel criteria for the diagnosis of bacterial vaginosis from Gram-stained vaginal smears, we first examined the reliability of each of the three components of the diagnosis and then the interpretation of bacterial vaginosis (Table 2). Although the smears were evaluated on a scale of 1 to 4+, the results for each component were first analyzed as present or absent, as described above. For the overall diagnosis of bacterial vaginosis, the correlation coefficient (0.60) showed moderate agreement between centers. Analysis of the three components used in the Spiegel criteria for bacterial vaginosis evaluated as present or absent showed moderate agreement for lactobacillus (0.64) and *G. vaginalis* (0.51) morphotypes and poor agreement for the summary variable of at least one other morphotype (0.27). When the other individual morphotypes making up the summary variable were analyzed, agreement was moderate for *Bacteroides* spp. morphotypes (0.53), excellent for *Mobiluncus* spp. morphotypes (0.83), and poor for gram-positive cocci (0.08).

Because more than one microbiologist was responsible for reading the smears at three of the five sites, we also examined the level of intracenter reliability for each of the morphotypes. All centers showed excellent levels of agreement for lactobacillus morphotypes (0.72 to 0.88) and curved gram-variable rods (0.73 to 0.99). Four of the centers had excellent agreement for *G. vaginalis* (0.81 to 0.86), with one center having poor agreement (0.45). Three centers had moderate to excellent agreement for *Bacteroides* spp. morphotypes (0.67, 0.86, 0.91), one center had poor agreement (0.31), and one center did not record *Bacteroides* spp. morphotypes, because they felt that it was impossible to differentiate *Gardnerella* spp. from *Bacteroides* spp. without culture results. Gram-positive cocci showed the greatest intracenter variability, with two centers having poor reliability (0.07, 0.41), two centers having moderate reliability (0.50, 0.64), and one center having excellent reliability (0.87). Centers with fewer microbiologists had consistently better intracenter agreement, as would be expected.

To understand the levels of agreement better, all five morphotypes were examined by using the Spearman rank correlation coefficients and paired *t* tests on the full 1 to 4+ scale rather than their presence or absence. For lactobacillus morphotypes, the agreement was similar (0.65) to the present or absent results, although it was clear from the mean differences that the reference center was recording significantly larger numbers of lactobacilli. *G. vaginalis* (0.69) and *Mobiluncus* spp. (0.85) morphotypes showed high correlations between the two readings, with small mean differences in the category scored indicating excellent intercenter agreement. The correlation coefficient for *Bacteroides* spp. morphotypes (0.57) indicated fair agreement between centers. However, the highly significant mean differences in organisms that were seen indicate that there were systematic differences in the interpretations between centers, indicating only fair or poor intercenter reliability. This difference may result, at least in part, from a reluctance of some centers to differentiate *G. vaginalis* from *Bacteroides* spp. morphotypes in the absence of culture results. A summary variable combining the two morphotypes as small gram-variable rods was created. The agreement for this variable (0.74) was excellent. Gram-positive cocci showed poor intercenter correlation (0.23) with highly significant mean differences, indicating poor intercenter reliability.

When the microbiologists first interpreted the Gram-stained smears, in addition to scoring the morphotypes on a 1 to 4+ scale, they were also asked to give their impression of the flora present by using three categories (normal, intermediate, bacterial vaginosis) rather than choosing only whether bacterial vaginosis was present or absent. The considerable debate over how to identify smears which were between normal and abnormal lead to an attempt to classify quantitatively the smears into a continuum by using the morphotypes observed rather than a dichotomy such as the Spiegel criteria. The three morphotypes shown to be most reliable in these analyses, *Lactobacillus* spp., *G. vaginalis*, and *Mobiluncus* spp., were used to create a 0- to 10-point scale, with normal being at the low end and bacterial vaginosis being at the upper end.

The total score was derived by summing the contributions of the individual morphotypes. Weights for the individual morphotypes were given (Table 1). The curved rod morphotype was given less weight because of its lower prevalence and the sense that it was seen as part of an end-stage process in patients with bacterial vaginosis. The presence of gram-positive cocci was not included as part of the score because it had the poorest agreement both within and between centers. The interpretations made by this scoring system showed an intercenter correlation of 0.82, with very small mean differences in the category scored, indicating excellent agreement.

DISCUSSION

This study evaluated the intercenter reliability of the criteria for diagnosing bacterial vaginosis and of the bacterial morphotypes which are components of the criteria. The goals of this evaluation were to determine the reasons that the Spiegel criteria for diagnosing bacterial vaginosis had only moderate intercenter agreement, to determine which bacterial morphotypes had the best intercenter reliability, and to use these morphotypes to devise a new scoring

system for bacterial vaginosis which provided for gradations in severity and had improved intercenter reliability.

The Spiegel criteria for diagnosing bacterial vaginosis had moderate intercenter agreement because it forced the diagnosis into broad categories of the presence or absence of bacterial vaginosis, which did not account for the spectrum of severity. The Spiegel criteria also depend on several bacterial morphotypes, *Bacteroides* spp. and gram-positive cocci, which had moderate to poor intercenter reliabilities. The moderate agreement for *Bacteroides* spp. morphotypes was partially because the microbiologists were unwilling to differentiate between *G. vaginalis* and *Bacteroides* spp. morphotypes in the absence of culture data. Had the centers been more comfortable in making these distinctions in the absence of culture results, the agreement may have been improved.

A standardized scoring system for the interpretation of Gram-stained vaginal smears has been proposed. The scoring system provides a 0- to 10-point scale for the evaluation of vaginal flora; the scale is based on a weighted sum of the following bacterial morphotypes with good to excellent intercenter reliability: lactobacilli, *G. vaginalis*, and *Mobiluncus* spp. The standardized score had improved intercenter reliability ($r = 0.82$) compared with the Spiegel criteria ($r = 0.61$).

The results of this study indicate that criteria for the diagnosis of bacterial vaginosis by using the Gram stain can be reproduced reliably between different centers and microbiologists. When the most reliable of the bacterial morphotypes are used to produce a summary score, that score can be used to assess the degree of alteration in vaginal flora as a continuum rather than as a forced dichotomy.

Bacterial vaginosis is an important genital syndrome because it affects a large number of women and because it has been associated with the pregnancy complications of amniotic fluid infection, prematurity, histologic chorioamnionitis, and postcesarean endometritis. Evaluation of the role of bacterial vaginosis in women with these pregnancy complications, although deserving further study, is hindered without a reliable, standardized test. Clinical signs are very difficult to standardize between clinicians and may be impossible to interpret during certain pregnancy situations such as labor. A previous report (9) has pointed out the predictive value of using Gram-stained vaginal smears rather than gas-liquid chromatography or vaginal cultures to identify women with bacterial vaginosis. The Gram-stained vaginal smear can be enhanced further by an expanded scale, by using the most reliable morphotypes, and by a standardized method of interpretation.

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