Evaluation of a Rapid Gram Stain Interpretation Method for Diagnosis of Bacterial Vaginosis

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Bacterial vaginosis is the most frequent cause of vaginitis (11) and has been associated with severe sequelae (8, 12). Symptoms of bacterial vaginosis are nonspecific, and diagnosis should rely on confirmatory tests (3, 6, 10). The two most widely accepted methods for the diagnosis of bacterial vaginosis, Amsel’s composite criteria (ACC) (2) and Nugent’s Gram stain evaluation of bacterial morphotypes (NBM) (9), are used insufficiently in routine practice (7, 14). The former is cumbersome and not easily subjected to quality control, and the apparent complexity of the latter may have limited its adoption by clinical laboratories. The aim of this study was to evaluate a simple interpretation of Gram-stained smears, described by Thomason et al. (Thomason’s clue cell criteria [TCC]) (13), against the two aforementioned methods for the diagnosis of bacterial vaginosis and to examine interobserver agreement for the Gram stain methods.

Patients and samples. Women who had a gynecological examination at the Department of Sexually Transmitted Infections at Landspítali University Hospital were recruited after written informed consent was obtained. A dry speculum was inserted into the vagina, and drops of fluid from the fornix were placed in 10% KOH for the amine odor test and on a pH indicator strip (Merck; pH range, 3.8 to 5.4). The presence of a white or gray homogenous discharge was recorded. Vaginal fluid was also sampled on a rayon swab with Amies agar gel (Copan Innovation), stored at 4 to 8°C, and processed within 24 h by rolling on glass slides for a wet mount and Gram staining. At least 20 fields of the wet mount were examined by one observer at ×400 magnification in a phase-contrast microscope (Leica DM LB) and the presence or absence of clue cells was recorded to complete the ACC method. The Gram-stained slide was evaluated by three observers according to the TCC method, with three modifications: (i) the slides were read at ×500 magnification with oil immersion, (ii) a microscopic field had to contain at least five epithelial cells to be considered valid, and (iii) lactobacilli were defined as any gram-positive, straight, or curved rods that had parallel sides and square ends and measured at least 2 μm in length (5). At least 20 fields were examined. Bacterial vaginosis was considered present if non-Lactobacillus morphotypes outnumbered Lactobacillus morphotypes and ≥2 clue cells were seen per 20 fields. The smears were also examined at ×1,000 magnification and scored according to the NBM method; intermediate status was classified as being negative for performance evaluation. Leica DM LB and Leitz Diaplan microscopes were used for regular microscopy. Results from each method and observer were blinded from other methods and observers. The study was approved by the institution’s Ethical Committee.

Analysis. The NBM method was used as the reference method for true-positive and -negative results. The sensitivities, specificities, positive and negative predictive values, and likelihood ratios for the TCC and ACC methods were calculated after resolving interobserver discordance for the TCC and NBM methods. A likelihood ratio of >10 was considered to provide strong evidence to rule in a diagnosis when positive results were obtained for a test (4). The intraobserver agreement between the TCC and NBM methods and the interobserver agreement for observer pairs’ results of both methods were calculated using each observer’s initial results. Kappa measure and Kendall’s correlation coefficient were used for agreement measures. Kappa values of 0.61 to 0.8 and 0.81 to 1.0 were considered to indicate “good” and “very good” strengths of agreement, respectively (1). Data were processed by the SPSS 11 software program (SPSS Inc., Chicago, IL).

Three hundred twenty-seven women were included; the median age was 22 years (range, 14 to 58 years). Bacterial vaginosis was diagnosed for 115 women by the NBM and TCC methods and for 106 by the ACC method. The performances of the TCC and ACC methods are shown in Table 1. The TCC method yielded false-negative results in 12 cases, all of which lacked the clue cell criterion, and false-positive results in 12 cases, all of which were graded as intermediate by the NBM method. Of the 22 samples that were positive by the TCC method and negative by the ACC method, 17 were shown to be true positives by the NBM method. Thirteen samples were negative by the TCC method and positive by the ACC method; nine of them had a negative or intermediary grade by the NBM method. The ACC method yielded false-negative results in 24.

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cases, most of which showed clue cells and raised pH but lacked the homogeneous discharge and whiff test criteria, and false-positive results in 7 cases, none of which showed clue cells. Analysis of the intraobserver agreement between the TCC and NBM methods yielded kappa values of 0.7, 0.85, and 0.86 for the three observers. The interobserver agreement analysis for the same methods yielded kappa values of 0.75, 0.78, and 0.94 for the TCC method and 0.83, 0.83, and 0.91 for the NBM method. Analysis of the individual components of the methods showed that the lowest correlation coefficients were obtained for the clue cell criterion (TCC) and the Lactobacillus score (NBM).

Our study is the first to assess the utility of the TCC method after the initial publication by Thomason et al. The method compared favorably with the currently accepted methods, despite the modifications made to it, and demonstrated high interobserver reliability. The performance of the TCC method as compared to the ACC method was, in this study, almost identical to that reported in the initial study of Thomason et al., a comparison of Thomason’s method to Amsel’s criteria (13). Intra- and interobserver disagreement was related mainly to the clue cell count in the TCC method and the Lactobacillus count in the NBM method. The observers who included partly covered epithelial cells with furry borders had a better agreement with the NBM method than the one who counted only completely covered cells, and the clue cell definition also explained the interobserver disagreement for the TCC method. As for the Lactobacillus score, interobserver disagreement was mainly seen for low Lactobacillus counts in the NBM method, due to the occasional difficulty in assigning a small gram-positive rod to the Lactobacillus morphotype. In the presence of abundant Gardnerella or Bacteroides morphotypes, the diagnosis of bacterial vaginosis by the NBM method depends on the exact and sometimes difficult scoring of the lowest Lactobacillus counts.

The rapid Gram stain evaluation is reliable, easy to perform, and well suited for the routine clinical laboratory. The results of our study indicate that further work on selected interpretative aspects of the Gram stain methods, especially the definition of clue cells and the scoring criteria for Lactobacillus morphotypes, is needed.

**REFERENCES**


**TABLE 1. Performance of TCC and ACC methods**

<table>
<thead>
<tr>
<th>Test method</th>
<th>Reference method</th>
<th>% Valuea</th>
<th>LRb (95% CI)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Sensitivity</td>
<td>Specificity</td>
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<tr>
<td>TCC</td>
<td>NBM</td>
<td>90</td>
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<tr>
<td></td>
<td>ACC</td>
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<tr>
<td>ACC</td>
<td>NBM</td>
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<td>93</td>
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a PPV, positive predictive value; NPV, negative predictive value.  
b LR, likelihood ratio.