DNA Hybridization Test: Rapid Diagnostic Tool for Excluding Bacterial Vaginosis in Pregnant Women with Symptoms Suggestive of Infection

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This prospective comparative study evaluated a DNA hybridization test (Affirm VPIII) as an alternative to Gram stain for the diagnosis of bacterial vaginosis. We examined vaginal smears from 1,725 pregnant women between the 12th and 36th weeks of gestation with clinical signs of vaginal infection. The DNA hybridization test compared well with Gram stain and can be used as a rapid diagnostic tool to exclude bacterial vaginosis.

Common reproductive tract infections and associated inflammatory responses are the most frequent gynecological complaints, representing a central problem in modern clinical care. Vaginitis is usually characterized by vaginal discharge, vulvar itching and irritation, or odor. Even though the causative microorganisms are manifold, the three diseases most frequently associated with vaginitis are bacterial vaginosis, vulvovaginal candidiasis, and trichomoniasis (3). The complications of bacterial vaginosis can be especially substantial in pregnant women, increasing the risk of an adverse pregnancy outcome, including histological chorioamnionitis (5), amniotic fluid infection (12), preterm labor, and preterm delivery (8, 9).

Laboratory methods for the identification of bacterial vaginosis include wet mount, Gram stain, the “gold standard” of diagnosis, and microbiological culture. Because microscopic evaluation by wet mount or Gram stain requires special diagnostic skills not available to all practitioners, overdiagnosis is common and therapy is frequently empirical. Although cultures for Gardnerella vaginalis constitute a highly sensitive method, they are not recommended for the diagnosis of bacterial vaginosis due to the high number of women positive for G. vaginalis who do not have clinical symptoms of bacterial vaginosis (2, 6).

Furthermore, determination of G. vaginalis by culture is rarely useful because of the relatively long period between the examination and the time the results are finally available. Because the different forms of vaginitis call for different treatment regimens, there is a need for a rapid and highly selective tool to help the care provider diagnose bacterial vaginosis and distinguish it from other types of infection. The aim of this study was to evaluate a DNA hybridization test (Affirm VPIII) as an alternative to Gram-stained microscopic slides, currently considered the most reliable diagnostic tool.

This study on the prospective comparison of two diagnostic tools included 1,725 pregnant women between the 12th and 36th weeks of gestation attending the outpatient clinic of the Department of Obstetrics and Gynecology at the University of Vienna, Vienna, Austria. If clinical symptoms such as increased vaginal discharge combined with pruritus and/or burning, cervical incompetence, lower abdominal pain, preterm labor, or preterm rupture of the membranes were present, two vaginal specimens were obtained with the Affirm VPIII sample collection set on Dacron tipped swabs for both Gram stain and nucleic acid hybridization. The samples were immediately transported to the laboratory of microbiology at our department. Physicians and medical technicians evaluated the Gram-stained microscopic slides. Interpretation was based on the Nugent scoring system (10), which we slightly modified to classify into four categories. Thus, grade 0 corresponds to a smear without any microbe, grade 1 is dominated by normal Lactobacillus (Nugent score, <4), grade 2 represents an intermediate flora (Nugent, 4 to 6), and grade 3 shows the flora typical of bacterial vaginosis (Nugent, >6) dominated by G. vaginalis and other anaerobic bacteria with a corresponding lack of Lactobacillus.

A medical technician processed the DNA hybridization test according to the manufacturer’s protocol. The DNA hybridization test (Affirm VPIII; Becton Dickinson and Company, Sparks, Md.) is a commercial system for the detection of G. vaginalis, Candida spp., and Trichomonas vaginalis appropriate for use in an outpatient setting. The test uses two distinct single-stranded nucleic acid probes for each organism as well as capture probes and color development probes that are complementary to unique genetic sequences of the target organisms. The capture probes are immobilized on a bead embedded in a probe analysis card, which contains a separate bead for each target organism. The procedure involves three steps: (i) sample preparation to release target organism nucleic acids, (ii) automated assay processing, and (iii) reading of the results after 30 min. The hybridization test includes a positive control and a negative bead on each probe analysis card, which are tested simultaneously with each patient specimen. The test was routinely performed for the detection of G. vaginalis, and its sensitivity was directly related to the Gram stain findings. The statistical measures for comparison of the Gram stain and Affirm test were sensitivity, specificity, positive and negative predictive values, and overall agreement with their respective 95% confidence intervals. A P value of <0.5 was considered to indicate statistical significance.

Vaginal swabs for both Gram stain and hybridization assay...
TABLE 1. Gram stain versus hybridization test in the detection of bacterial vaginosis: grades 0 and 1 versus grades 2 and 3 according to the modified Nugent criteria

<table>
<thead>
<tr>
<th>Grade</th>
<th>Total no. of smears</th>
<th>Hybridization test</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of G. vaginalis negative</td>
<td>No. G. vaginalis positive</td>
<td></td>
</tr>
<tr>
<td>0 and 1</td>
<td>1,319</td>
<td>1,281</td>
<td>38</td>
</tr>
<tr>
<td>2 and 3</td>
<td>406</td>
<td>109</td>
<td>297</td>
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<tr>
<td>Total</td>
<td>1,725</td>
<td>1,390</td>
<td>335</td>
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</table>

* Sensitivity, 73.2% ± 2.2% (68.8–77.5%); specificity, 97.1% ± 0.5% (96.2–98.0%); positive predictive value, 88.7% ± 1.7% (85.3–92.1%); negative predictive value, 92.2% ± 0.7% (90.7–93.6%); accuracy, 91.5% ± 0.7% (90.2–92.8%).

TABLE 2. Gram stain versus hybridization test in the detection of bacterial vaginosis: grades 0 and 1 versus grade 3 according to the modified Nugent criteria

<table>
<thead>
<tr>
<th>Grade</th>
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<th>Hybridization test</th>
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<tr>
<td>0 and 1</td>
<td>1,316</td>
<td>1,278</td>
<td>38</td>
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<tr>
<td>3</td>
<td>171</td>
<td>18</td>
<td>153</td>
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<tr>
<td>Total</td>
<td>1,487</td>
<td>1,296</td>
<td>191</td>
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</table>

* Sensitivity, 89.5% ± 2.3% (84.9–94.1%); specificity, 97.1% ± 0.5% (96.2–98.0%); positive predictive value, 80.1% ± 2.9% (74.4–85.8%); negative predictive value, 98.6% ± 0.3% (98.0–99.2%); accuracy, 96.2% ± 0.5% (95.3–97.2%).

The purpose of this investigation was to evaluate the Affirm VPIII Microbial Identification Test as a possible replacement for microscope examination in clinical settings where immediate microscopic examination of specimens is not feasible. Of the methods available for laboratory confirmation of bacterial vaginosis, vaginal Gram stain is faster and more useful than culture (4). Due to its low specificity, vaginal culture has no part in the diagnosis of bacterial vaginosis, which is why we did not assess the value of the hybridization test in comparison to culture findings.

Because of its high specificity (97.1%), the Affirm test is an excellent tool for diagnosing the presence of bacterial vaginosis, which may be associated with a possibly increased risk of preterm birth. With regard to sensitivity, the test can be interpreted from two different viewpoints. Although the sensitivity for grade 2 and 3 cases according to the modified Nugent criteria is only 73.2%, the therapeutic relevance of an intermediate flora (grade 2) in day-to-day clinical care is somewhat hazy. The Affirm test does not yield a positive result for germ counts below $2 \times 10^5$, which ensures that only clinically relevant cases of infection are detected, thereby avoiding overinterpretation and overtreatment. Therefore, when grade 2 cases, whose clinical relevance is unclear, are dropped from the analysis, test sensitivity improves significantly, to 89.5% ($P < 0.00001$), and the high specificity remains unchanged, with these values comparing well with those obtained in other reports (1). Viewed from this perspective, the test provides an excellent tool for the diagnosis or exclusion of bacterial vaginosis.

Although Gram’s staining of vaginal secretions is even more reliable than wet mount, with a sensitivity of 93% and specificity of 70%, it is underused, apparently because only a few clinicians are adequately trained to use microscopy (10). The Affirm test does not yield positive results with organism counts below $2 \times 10^5$ for G. vaginalis, reducing the probability of false-positive results. This helps limit treatment to positive patients, facilitating more cost-effective use of health-care resources. The cost reductions thus effected most likely compensate for the acquisition and marginal costs of performing the test. Moreover, the specific detection of relevant organisms ensures a beneficial cost-benefit ratio compared with culture and the length of time involved until test results are available. With an adequate organizational infrastructure in place, Affirm test results can be obtained within 40 min and treatment can be started on the day of testing.

In summary, the Affirm VPIII DNA hybridization test provides an easy and automated way for diagnosing bacterial vaginosis. Our results show that the test compares well with Gram stain and can be used as a rapid diagnostic tool to exclude bacterial vaginosis in pregnant women. An extensive body of evidence indicates that there is an increased risk of preterm birth in women with bacterial vaginosis (7, 8, 11). Even though recommendations for routine screening and treatment still await the results of further interventional studies, routine screening may become the standard of care. In this case, physicians will need a reliable, rapid, point-of-care screening tool, and the hybridization test fulfills all of these criteria.

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REFERENCES


