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High Levels of *Gardnerella vaginalis* Detected with an Oligonucleotide Probe Combined with Elevated pH as a Diagnostic Indicator of Bacterial Vaginosis

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We have demonstrated a new approach to diagnosing bacterial vaginosis (BV) that is based on measuring the concentration of *Gardnerella vaginalis* in vaginal fluid with DNA probes. *G. vaginalis* is virtually always present at high concentrations in women who have BV but is also detected frequently in normal women, usually at concentrations of $<10^7$ CFU/ml of vaginal fluid. Elevated vaginal pH is another sensitive indicator of BV, although it can occur in conjunction with other conditions. We have proposed that quantitative measurements of *G. vaginalis* using specific DNA probes can serve as a useful aid in diagnosing BV, provided the vaginal pH is above 4.5. To test this hypothesis, a group of 113 women were first evaluated for BV by the standard set of clinical signs. Vaginal washes were collected, and aliquots were analyzed by quantitative culture for the concentration of *G. vaginalis*. Portions of these same samples were immobilized on nylon filters, along with standards for quantitation. The filters were incubated with a radiolabelled oligonucleotide specific for *G. vaginalis* 16S rRNA, and the subsequent autoradiographs were examined to determine levels of *G. vaginalis* in each sample. *G. vaginalis* at concentrations of $\geq 2 \times 10^7$ CFU/ml and vaginal pH of >4.5 were then analyzed for concurrence with the diagnoses based on clinical criteria. Results of this slot blot analysis gave a sensitivity of 95%, correctly categorizing 41 of 43 BV-positive specimens, and a specificity of 99%, correctly identifying 69 of 70 BV-negative specimens, compared with diagnosis based on clinical criteria.

Bacterial vaginosis (BV), formerly known as nonspecific vaginitis, is a condition characterized by a decrease in vaginal lactobacilli, and a dramatic increase in the numbers of other microorganisms present, especially *Gardnerella vaginalis*, genital mycoplasmas, *Mobiluncus* spp., and other anaerobes (10, 28). Women seeking medical attention for BV are usually prompted to do so by a vaginal discharge and/or an unpleasant odor (16). Despite its mild clinical presentation, numerous studies have shown a consistent association between BV and premature births (6, 17, 19), chorioamnionitis (12), amnionic fluid infections (9, 27), and postcesarean endometritis (6, 34). Accordingly, the importance of diagnosing BV in women of childbearing age is gaining recognition.

G. vaginalis was originally considered to be the etiologic agent of BV when the organism was discovered in association with a white vaginal discharge and found to be lacking in normal women (8). However, subsequent studies have revealed the presence of *G. vaginalis* in up to 60% of normal women (7, 18). Additional studies have shown that *Bacteroides* spp., *Mobiluncus* spp., and *Mycoplasma hominis* are also associated with BV, although none of these alone necessarily indicates the disease (14). Therefore, a standard diagnostic protocol for BV has been developed that relies on clinical signs rather than the classical method of microbiological culture (1). Other methods for diagnosing BV have included analyzing Gram stains of vaginal smears (5, 21, 29), gas chromatographic analysis of fatty acids present in vaginal secretions (28), and a test for the presence of proline aminopeptidase (31).

Although *G. vaginalis* is found in a large proportion of BV-free women, quantitative culture has indicated that the overall levels are higher for BV-positive women, even though there is considerable overlap in *G. vaginalis* levels between BV-negative and -positive groups (1, 32, 33). Nearly all BV-positive women are culture positive for *G. vaginalis* (3, 11, 23) at a median concentration of 10^9 CFU/ml of vaginal fluid (11). Similarly, elevated vaginal pH is characteristically present in BV-positive women, but elevated pH can also result from cervicitis, trichomoniasis, or recent sexual intercourse (7, 30). In this study, we have proposed and tested the hypothesis that finding high levels of *G. vaginalis* can aid in diagnosing BV, provided that elevated vaginal pH is considered simultaneously to be a criterion for a positive diagnosis.

To test this hypothesis, an oligonucleotide probe has been developed that hybridizes specifically with the 16S rRNA of *G. vaginalis*. Pao et al. (22) have developed *G. vaginalis*-specific DNA probes for detecting single-copy DNA in the genome of *G. vaginalis*, but the approach presented here offers the advantage of detecting a highly abundant cellular component (rRNA) that is present at several thousand copies per cell.

In this study, vaginal washes were collected from 43 women who were positive for BV by standard clinical criteria and from 70 women who were negative for BV. All 113 samples were analyzed with oligonucleotide probes on slot blots for the level of *G. vaginalis*. Samples from most of these patients were analyzed also by quantitative culture for *G. vaginalis*.

MATERIALS AND METHODS

Patient samples. Vaginal washes were obtained from women with a mean age of 23.8 years who were attending the

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Student Health Clinic at the University of Washington. Eighty percent were unmarried, and none were pregnant. To minimize unintended variables, sample donors were instructed not to douche, use spermicide, or engage in sexual intercourse for 24 h prior to their clinic appointment. Samples for the BV-negative group were taken from women who were having an annual examination. Women who were positive by wet mount for *Trichomonas vaginalis* or *Candida* spp. were excluded from the study. The BV-positive group consisted of women who presented with at least three of the following four signs: (i) clue cells upon microscopic examination, (ii) homogeneous white or gray adherent discharge, (iii) vaginal fluid with a pH of >4.5, and (iv) amine odor following the addition of 10% KOH (1).

To collect samples, 3 ml of a sterile balanced salt solution (17) was injected into the vagina, withdrawn, and placed in a sterile test tube. Such vaginal wash samples have been determined to be roughly equivalent to twofold dilutions of vaginal fluid (11). For quantitative culture, a portion of each vaginal wash was used to prepare serial 1:10 dilutions, each of which was inoculated onto human bilayer Tween 80 agar, and incubated for 48 h at 37°C (33). *G. vaginalis* was identified in cultures on the basis of beta hemolysis, negative catalase reaction, and Gram stain (24). Culture results were expressed as CFU per milliliter of undiluted vaginal fluid. Cultures were not performed on 35 of the samples. After aliquots had been removed for culture, 0.25 ml of each vaginal wash was added to 0.5 ml of lysis solution containing 4.5 M guanidinium thiocyanate, 5% formamide, 2% *N*-laurylsarcosine, 50 mM Tris (pH 7.5), and 10 mM EDTA; lysed samples were stored at −70°C until used for slot blots.

Probe design. Total nucleic acid (TNA) from *G. vaginalis* (ATCC 14018) was extracted with phenol-chloroform (25) and used as a template for dideoxynucleotide sequencing (15, 26). Primers for sequencing were oligonucleotides corresponding to conserved regions of bacterial 16S rRNA that lie adjacent to hypervariable regions (15). On the basis of the RNA sequence thus determined, several 24-base oligonucleotide probes were designed for maximum species specificity, using the strategy previously described (4). Oligonucleotides were synthesized on an Applied Biosystems 380B DNA synthesizer (Foster City, Calif.) by beta-cyanoethyl phosphoramidite chemistry, and purified by high-pressure liquid chromatography (1a).

Slot blots. For specificity testing, microorganisms were obtained from the American Type Culture Collection (Table 1) and propagated in culture, and the TNA was extracted with phenol-chloroform (25). One microgram of each TNA sample was loaded onto Nytran filters (Schleicher and Schuell, Keene, N.H.) using a Schleicher and Schuell Minifold II slot blot template. Serially diluted 16S rRNA from *G. vaginalis* was included on each filter as a standard for quantitation, as described more fully below. The 16S rRNA was purified by hybridization of *G. vaginalis* TNA with a biotinylated oligonucleotide complementary to the 16S rRNA and binding of the hybrid to streptavidin-conjugated agarose beads purchased from Pierce (Rockford, Ill.). The biotinylated oligonucleotide used for this purpose was UP041, whose sequence is given in the Results. The 16S rRNA was eluted from the beads by heating in sterile water to 85°C for 5 min, and the released rRNA was precipitated with ethanol. The purity of the 16S rRNA was verified by agarose gel electrophoresis.

Patient samples were prepared for slot blots as described above, and cultured *G. vaginalis* was prepared by lysis of pelleted bacteria in a solution of 3 M guanidinium thiocyanate, 5% formamide, 2% *N*-laurylsarcosine, 50 mM Tris (pH 7.5), and 10 mM EDTA. Samples were suspended in lysis solution, heated for 2 min at 65°C, and filtered through Nytran filters. After application of samples, filters were rinsed briefly in 2× SSC (1× SSC is 0.15 M sodium chloride plus 0.015 M trisodium citrate [pH 7.0]) and baked at 80°C for 2 h. Before hybridization, some of the filters were bisected along a line parallel and central to each row of slots, thus dividing each slot in half; this was done so that each half-row could be hybridized separately. Oligonucleotides to be tested were labelled with terminal deoxynucleotidyl transferase obtained from U.S. Biochemicals (Cleveland, Ohio), using the substrate [^{32}P]3′-deoxyadenosine 5′-triphosphate (Cordycepin 5′-triphosphate) with a specific activity of 5,000 Ci/mmol (New England Nuclear, Boston, Mass.). Reactions were conducted as described in the U.S. Biochemicals product insert that accompanied the terminal deoxynucleotidyl transferase. Filters to which nucleic acid had been fixed were hybridized overnight at 42°C with 10^6 cpm/ml of ^{32}P-labelled oligonucleotide. The hybridization buffer consisted of 0.6 M NaCl, 90 mM Tris (pH 8.0), 10 mM EDTA, 0.5% sodium dodecyl sulfate (SDS), 5× Denhardt's solution, 30% deionized formamide, and 100 μg of hydrolyzed yeast RNA per ml. After hybridization, filters were washed three times at room temperature in a solution of 90 mM NaCl, 9 mM Tris (pH 8.0), 0.6 mM EDTA, and 0.2% SDS and exposed to Kodak X-OMAT AR film (Rochester, N.Y.).

Calibration of standards for slot blots. To analyze patient samples, slot blot results were expressed as numbers of *G. vaginalis* cells per milliliter of vaginal fluid so that slot blot results could be compared directly with culture results, which were expressed as CFU per milliliter of vaginal fluid. Biochemically pure 16S rRNA provided the basis for the conversion of slot blot signals to numbers of *G. vaginalis* per milliliter of vaginal fluid.

Experiments were conducted to determine the approximate number of 16S rRNA molecules present in a given quantity of *G. vaginalis* cells. Slot blots were prepared that contained both serially diluted 16S rRNA and serially diluted lysates made from exponentially growing cultures of *G. vaginalis* for which titers had been determined by using a Petroff-Hauser chamber to microscopically count bacterial cells. This method of counting is independent of cell viability. Before preparing these slot blots, control experiments were conducted whose results showed that crude bacterial lysates (as opposed to phenol-extracted nucleic acids) on the slot blot filters did not significantly affect the hybridization results. To calibrate the standards, three different cultures of *G. vaginalis* were counted, and serially diluted lysates of each culture were applied in duplicate rows to slot blots for hybridization with ^{32}P-labelled oligonucleotide. An autoradiograph of one of these slot blots is shown in Fig. 1. In the example shown in Fig. 1, comparison of the signals in panels A and B indicated that 1 ng of 16S rRNA was the equivalent of about 5×10^5 lysed *G. vaginalis* cells. By averaging the values obtained from all six slot blot comparisons, 1 ng of 16S rRNA was equated with 5×10^5 cells ($\pm 2.9 \times 10^5$ cells), or about 2,400 copies of 16S rRNA per cell. In clinical specimens, the amount of 16S rRNA per cell is likely to vary as a function of stage in the *G. vaginalis* growth cycle, but this parameter was not accessible to evaluation.

Evaluation of autoradiograms for clinical sample analysis. Signals on autoradiograms were compared visually with the 16S rRNA standards. Two different investigators read each film. Differences having a magnitude of two- to threefold

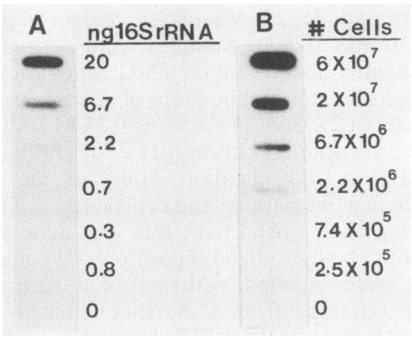

FIG. 1. Comparison on slot blots of *G. vaginalis* 16S rRNA and *G. vaginalis* lysates. Serially diluted purified 16S rRNA from *G. vaginalis* and serially diluted lysates of cultured *G. vaginalis* were immobilized on Nytran filters. Prior to lysis, the optical density of cell cultures at 600 nm was read, and optical density readings were multiplied by 10^9 organisms per ml. Lysates were prepared by suspending pelleted bacteria in a small volume of 3.0 M guanidinium thiocyanate. Lysates were stored frozen until being applied to filters as described in Materials and Methods. Filters were hybridized with ^{32}P-labelled GV003.

were seen on occasion between the values determined by the two investigators. In such cases, the differences were resolved by careful reexamination of the X-ray film. Based on such readings, levels of *G. vaginalis* were calculated without reference to the clinical diagnoses or culture results. In some instances, duplicate filters were hybridized with the same probe; autoradiograms produced by these identical filters were virtually indistinguishable.

RESULTS

Testing synthetic oligonucleotide probes for specificity. Several oligonucleotides complementary to *G. vaginalis* 16S rRNA sequences were synthesized and screened against TNA from each of the organisms listed in Table 1. This list includes representatives of all major groups of microorganisms that have been reported to be in the vagina or on the skin (2, 13, 20, 24, 32).

Before testing the *G. vaginalis* probes, the nucleic acids extracted from the organisms listed in Table 1 were validated by two kinds of control procedures. First, each TNA was examined on an agarose gel to ensure that the rRNA was intact. Next, an aliquot of each of the TNAs was immobilized on a slot blot filter, and this filter was hybridized with ^{32}P-labelled UP041 (5′ CTGCTGCCTCCCGTAGGAGT 3′), an oligonucleotide corresponding to a highly conserved (universal) sequence found in bacterial 16S rRNA. As expected, hybridization with UP041 yielded signals with all of the bacterial samples, but not with the eucaryotic TNAs present on the filter. This result (not shown) confirmed that hybridizable rRNA was present in all of the bacterial TNA preparations.

These TNAs were then immobilized on several replica filters, and each *G. vaginalis* oligonucleotide was labelled with ^{32}P and incubated with one of the replica filters. Hybridization results indicated that GV003 (5′ AGACGGC TCCATCCCAAAAGGGTT 3′) provided the required specificity, as it hybridized with only TNA extracted from *G. vaginalis* (results not shown).

To ascertain whether GV003 could be expected to detect a wide variety of clinical strains, lysates from 30 clinical isolates representing seven biotypes of *G. vaginalis* were

TABLE 1. Specificity panel

Strain
Acinetobacter calcoaceticus ATCC 19606
Actinobacillus actinomycetemcomitans ATCC 33384
Actinomyces viscosus ATCC 15987
Bacteroides fragilis ATCC 25285
Bacteroides gracilis ATCC 33236
Bacteroides multiacidus ATCC 27723
Bacteroides ureolyticus ATCC 33387
Campylobacter coli ATCC 33559
Campylobacter pylori ATCC 43504
Candida albicans ATCC 18804
Chlamydia trachomatis type K VR887
Clostridium difficile ATCC 9689
Clostridium perfringens ATCC 13124
Corynebacterium minutissimum ATCC 23349
Corynebacterium sp. ATCC 17892
Eikenella corrodens ATCC 23834
Escherichia coli ATCC 11775
Eubacterium lentum ATCC 25559
Fusobacterium mortiferum ATCC 25557
Gardnerella vaginalis ATCC 14018
Haemophilus aphrophilus ATCC 33389
Haemophilus paraphrophilus ATCC 29241
Haemophilus segnis ATCC 33393
Klebsiella pneumoniae ATCC 13883
Lactobacillus acidophilus ATCC 4356
Lactobacillus casei subsp. *rhamnosus* ATCC 7469
Lactobacillus catenaformis ATCC 25536
Lactobacillus jensenii ATCC 25258
Listeria monocytogenes ATCC 15313
Micrococcus luteus ATCC 323262
Mobiluncus curtisii subsp. *curtisii* ATCC 35241
Mobiluncus curtisii subsp. *holmeseii* ATCC 35242
Mobiluncus mulieris ATCC 35243
Mycoplasma hominis ATCC 23114
Neisseria cinerea ATCC 14685
Neisseria gonorrhoeae ATCC 19424
Neisseria lactamica ATCC 23970
Neisseria meningitidis serotype B ATCC 13090
Peptostreptococcus anaerobius ATCC 27337
Peptostreptococcus asaccharolyticus ATCC 14963
Peptostreptococcus magnus ATCC 15794
Peptostreptococcus tetradius ATCC 35098
Porphyromonas asaccharolytica ATCC 25260
Prevotella bivia ATCC 29303
Prevotella buccae ATCC 33574
Prevotella corporis ATCC 33547
Prevotella denticola ATCC 35308
Prevotella intermedia I ATCC 25611
Prevotella intermedia II ATCC 33563
Prevotella loescheii ATCC 15930
Prevotella melaninogenica ATCC 25845
Prevotella oris ATCC 33573
Prevotella veroralis ATCC 33779
Propionibacterium acnes ATCC 6919
Proteus mirabilis ATCC 29906
Proteus vulgaris ATCC 13315
Pseudomonas aeruginosa ATCC 10145
Staphylococcus aureus ATCC 12600
Staphylococcus epidermidis ATCC 14990
Staphylococcus saprophyticus ATCC 15305
Streptococcus agalactiae ATCC 13813
Streptococcus faecalis ATCC 19433
Streptococcus mutans ATCC 25175
Trichomonas vaginalis ATCC 30001
Ureaplasma urealyticum ATCC 27618
Veillonella parvula ATCC 10790
Wolinella curva ATCC 35224
Wolinella recta ATCC 33238
Wolinella succinogenes ATCC 29543
Ca Ski (human cell line) ATCC CRL1550

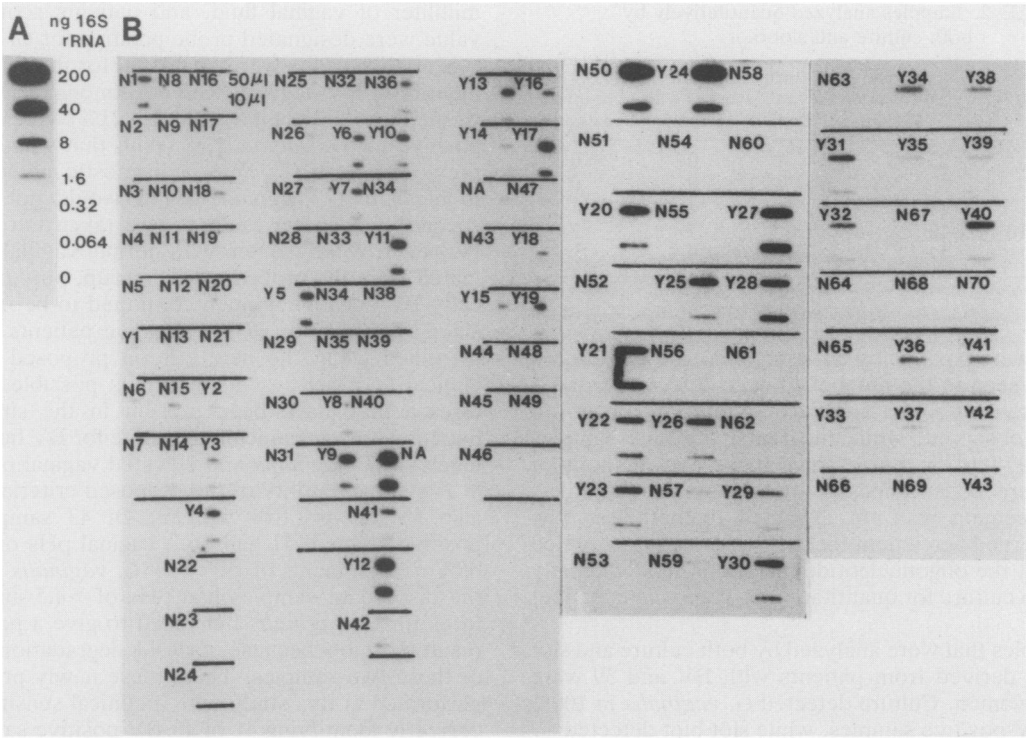

FIG. 2. Slot blot analysis of patient samples. Vaginal washes were collected and prepared for analysis as described in Materials and Methods. Fifty- and 10-μl aliquots of each patient sample were adjusted to a volume of 200 μl with 3 M guanidinium thiocyanate and loaded onto Nytran filters as described in Materials and Methods. Serially diluted 16S from *G. vaginalis* was loaded onto these same filters to provide standards for quantitation. Some filters were bisected, and only half of such filters were included in the hybridization, thus some of the slots appearing in the autoradiogram are only half the width of the others. Filters were hybridized with ^{32}P-labelled GV003. The numbers refer to individual patients; an N preceding the number indicates that the patient was BV negative by clinical criteria, while Y indicates that the patient was BV positive.

hybridized on slot blots with this probe. Cultured bacteria were lysed with guanidinium thiocyanate and then immobilized on duplicate slot blots. One of the filters was hybridized with labelled GV003, while as a control the other filter was hybridized with the universal probe, UP041. All of the clinical isolates reacted with GV003, and the filter probed with UP041 yielded an array of signal intensities that were indistinguishable from those produced by GV003. This result (not shown) indicated that all of the clinical isolates reacted equally well with the *G. vaginalis*-specific probe and suggests that GV003 can be expected to hybridize with rRNA from most if not all isolates of *G. vaginalis*.

Testing slot blot hybridization for reproducibility. Reproducibility of slot blots in the presence of patient samples was ascertained by analyzing filters that received patient samples spiked with known amounts (10^8, 5×10^6, or 10^5) of *G. vaginalis*. From each of six normal women, four vaginal swabs were collected and placed in lysis solution. Eluates from each group of four swabs were pooled and used as diluents for the three 25-fold dilutions of lysed cultured *G. vaginalis*. Swab eluate with no *G. vaginalis* added and *G. vaginalis* lysates diluted into lysis solution without any swab eluate were used as controls. The standard was purified *G. vaginalis* 16S rRNA. In comparing *G. vaginalis* lysates mixed with vaginal swab eluate to those without eluate, only 2 of the 15 slots containing swab eluate differed in signal intensity from the controls that received no eluate. These observed differences were less than fivefold in magnitude, suggesting the possibility that material present on some vaginal swabs could slightly alter the signal seen on slot blots. However, since the clinical study described below involved two dilutions of each patient sample, patient misclassification resulting from this low degree of sample interference seems unlikely.

Analysis of patient samples using slot blots. For slot blot analysis, serially diluted 16S rRNA from *G. vaginalis* and 50- and 10-μl aliquots of each patient sample were immobilized on Nytran filters, and hybridized with ^{32}P-labelled GV003, using the same hybridization conditions described above for the specificity tests. Figure 2A shows the autoradiogram of the 16S rRNA standards that were hybridized concurrently with the patient samples. In this experiment, the lower limit of visual detection for the 16S rRNA standard was about 0.32 ng, or the equivalent of about 1.6×10^5 cells.

The data depicted in Fig. 2 were analyzed to yield values for *G. vaginalis* levels in each patient sample. In comparison with the 16S rRNA standards, the signals observed in Fig. 2B were converted to the numbers of *G. vaginalis* cells per slot, and these numbers were normalized to the numbers of *G. vaginalis* per milliliter of vaginal fluid.

Slot blot results, which measured the number of cells per milliliter, compared favorably with culture results, which measured CFU per milliliter. Of the 113 samples, 78 were analyzed by both slot blot and culture. Of those 78, 17 samples were negative by both methods and 43 were positive by both methods. For 25 of the 43 samples positive by both methods, less than a 10-fold difference was observed in the number of cells detected by the two methods. For three

TABLE 2. Samples analyzed quantitatively by both culture and slot blot

Culture results (*G. vaginalis* CFU/ml)	No. of cultures	No. of slot blot samples with $\geq 2 \times 10^7$/ml	No. of samples with pH >4.5	No. of BV-positive patients
$<10^2$	23	2	0	0
$10^2 < n < 10^4$	3	0	0	0
$10^4 \leq n < 10^6$	10	6	4	4
$10^6 \leq n < 2 \times 10^7$	10	5	7	7
$n \geq 2 \times 10^7$	32	32	29	28

samples that were positive by both methods, a 10- to 20-fold variation was seen in the number of cells detected. For six samples positive by both methods, differences of 20- to 70-fold were observed, while the remaining nine samples differed by 100-fold or more. Since these two methods of quantitation are constrained by quite different variables, complete agreement was not expected. Nonetheless, the extent of observed agreement for the two methods provided assurance that the oligonucleotide method is fundamentally comparable to culture for quantitating *G. vaginalis* in patient samples.

Of the samples that were analyzed by both culture and slot blot, 39 were derived from patients with BV and 39 were from normal women. Culture detected *G. vaginalis* in 100% of these 39 BV-positive samples, while slot blot detected *G. vaginalis* in 37 (95%) of the 39. For the two samples that gave discrepant slot blot results, failure to hybridize may have resulted from degradation of the target rRNA. For the 39 BV-negative samples, culture was positive for 41% and slot blot was positive for 31% of the samples. Sixteen of the BV-negative samples contained detectable *G. vaginalis* by only one of the two methods. Apparently, culture and slot blot are both very effective in detecting *G. vaginalis* when high concentrations are present, as in BV-positive patients, while neither method is entirely reliable for detecting the low levels found in BV-negative women.

Table 2 compares the results of quantitative culture with those slot blot results in which high levels of *G. vaginalis* ($\geq 2 \times 10^7$/ml) were detected. Table 2 illustrates that slot blot detected high levels of *G. vaginalis* most often in those samples that contained high titers according to the culture method. More specifically, slot blot detected $\geq 2 \times 10^7$ cells per ml of vaginal fluid in all 32 of the samples whose cultures indicated $\geq 2 \times 10^7$ CFU/ml. Of the 20 samples for which culture detected 10^4 to 2×10^7 CFU/ml, 11 contained $\geq 2 \times 10^7$/ml according to slot blot. Of the samples shown in Table 2, 23 were culture negative; of these, 2 contained $\geq 2 \times 10^7$ of *G. vaginalis* per ml when measured by slot blot, a result possibly reflecting culture failure due to loss of viability during transport.

Diagnostic utility of *G. vaginalis* concentration and pH. Table 2, in addition to comparing slot blot with culture results, also illustrates the relationships observed between culture results, elevated pH, and BV-positive diagnosis. Elevated pH was observed only in samples with *G. vaginalis* titers of $\geq 10^4$ CFU/ml. Similarly, all of the samples from BV-positive patients had *G. vaginalis* titers of $\geq 10^4$ CFU/ml.

The values for *G. vaginalis* per milliliter measured by slot blot were determined for all 113 samples in the study group. This set of numbers was analyzed to determine the cutoff value that best discriminated between the clinically BV-positive and -negative groups. The best fit was obtained when the cutoff value was set at $\geq 2 \times 10^7$ *G. vaginalis* per milliliter of vaginal fluid, and patients scoring above this value were designated probe positive for *G. vaginalis*.

Slot blot results were evaluated for their clinical utility in identifying BV-negative women. Among the 70 samples from women who did not have BV, 15 (21%) were scored as probe positive for *G. vaginalis*, a result that was consistent with previous reports (7). Thus, a diagnostic method using probes to measure *G. vaginalis* levels would not be sufficiently specific if no other factors were taken into consideration. However, when women with normal vaginal pH were eliminated from the probe-positive group, only one of the clinically BV-negative women continued to be incorrectly classified. Thus, 69 of 70 BV-negative patients were correctly identified using the new criteria proposed here, giving a clinical specificity of 98.6%. It is possible that low-grade disease may have been present in the single discordant patient who was clinically negative for BV but who had high levels of *G. vaginalis* and elevated vaginal pH.

The clinical utility of the proposed criteria was evaluated also for BV-positive women. Of 43 samples from BV-positive women, 41 had both vaginal pHs of >4.5 and slot blot measurements of $\geq 2 \times 10^7$ *G. vaginalis* per ml. Both of the discordant samples had pHs of >4.5 and positive cultures for *G. vaginalis* but failed to give a positive slot blot result, possibly because of rRNA degradation during storage of these two samples. Thus, these newly proposed criteria performed in this study with a clinical sensitivity of 95.3%, correctly identifying 41 of 43 BV-positive samples.

DISCUSSION

The accepted "gold standard" for diagnosis of BV requires the evaluation of four clinical signs (1). Although the adoption of these uniform criteria has been invaluable in studies investigating the sequelae of BV, this approach has disadvantages with regard to routine use in the physician's office. For example, evaluating the appearance of vaginal discharge involves an inevitable element of subjectivity. Furthermore, the most significant of the clinical signs is the microscopic detection of clue cells. This time-consuming step requires the identification of characteristic vaginal epithelial cells that are covered with adherent small rod-shaped bacteria. Correct identification of clue cells is complicated by the fact that normal vaginal bacteria or debris sometimes adhere to vaginal keratinocytes, which thus masquerade as clue cells. Special training is required to distinguish genuine clue cells from these imposters. An objective pathogen-based method for diagnosing BV, one that did not require special skills, could assist physicians in diagnosing this disease that poses such a serious threat to women during pregnancy.

It seemed possible to us that simultaneous consideration of pH and *G. vaginalis* levels could serve as a means of identifying patients likely to have BV. Although *G. vaginalis* has been implicated in the etiology of bacterial vaginosis, culture of this organism has been of limited clinical utility because *G. vaginalis* so frequently occurs in normal women (1, 10, 28, 33). However, some studies have suggested that the levels of *G. vaginalis* are significantly higher in BV-positive patients (1, 11, 28, 33). Furthermore, while a vaginal pH of >4.5 is one of the clinical signs indicative of BV, this parameter cannot be used as the sole diagnostic indicator because elevated pH is a highly sensitive but nonspecific indicator for BV (7, 30). The results presented here demonstrate that considering pH together with *G. vaginalis* levels can be useful in diagnosing BV.

This study was designed to test the hypothesis that BV-positive and -negative women could be distinguished by measuring *G. vaginalis* levels and pH and was not intended to address the consequences of applying such a test to a random sample of women. One could speculate that if a BV-free woman with a high level of *G. vaginalis* were also infected with *T. vaginalis*, the *T. vaginalis* itself could result in elevated vaginal pH. According to the criteria proposed here, such a patient would appear to have BV even if she did not. In a preliminary study of trichomoniasis patients, slot blot analysis for *G. vaginalis* levels was performed on 12 samples from patients culture positive for *T. vaginalis*. Ten of these women had vaginal pHs of >4.5. Of these 10, only 3 appeared positive for BV according to the criteria of high vaginal pH and *G. vaginalis* levels. These three patients could have been false positives for BV, or they may have had BV concurrent with trichomoniasis. Information on these patients' BV status was not available. However, it is notable that 9 of the 12 *T. vaginalis*-positive samples analyzed did not score as positive for BV according to the proposed criteria. This result, although based on a limited sample size, suggests that false positives among *T. vaginalis*-infected women may be uncommon. The addition of a *T. vaginalis*-specific probe to the assay is possible and would further aid in distinguishing BV from trichomoniasis patients. Candidiasis would not be expected to produce false positives according to the proposed test for BV, as candidiasis does not cause elevated vaginal pH.

Although quantification of *G. vaginalis* may be accomplished by microbiological culture, this time-consuming procedure involves plating multiple serial dilutions and is rarely performed outside of research settings. Quantitative culture is not routine in most clinical microbiology laboratories, since for most diseases, only the absence or presence of a given pathogen has diagnostic significance. Specific oligonucleotide probes complementary to rRNA offer an alternative and objective means of rapidly quantitating pathogenic bacteria in a diagnostic setting. In fact, analysis by hybridization may be more intrinsically accurate, because it does not rely on viability or physiological condition of the organisms and is not affected by clumping of bacterial cells. Additional advantages of using probes rather than culture are that probes can be configured into an automated test requiring no special training, samples for probe analysis can be stored frozen until needed, and samples can be kept for repeated analyses at a later time.

The results of this study indicated an excellent agreement between the proposed criteria (high pH and high *G. vaginalis*) and a diagnosis of BV by the gold standard method. For this group of samples, which did not include candidiasis or trichomoniasis patients, using the probe and pH as criteria provided a sensitivity of 95.3% and a specificity of 98.6%. Although radioisotopes are not practical in a clinical setting, enzymatically tagged probes for the detection of *G. vaginalis* could be configured into a test suitable for use in the physician's office. The envisioned test could provide a useful adjunct to the standard physical examination of pregnant women or those presenting with vaginal symptoms.

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